

# **Integrating Above and Belowground Components of Biodiversity across Spatial Scales: The Role of Host Plants in the Distribution of Arbuscular Mycorrhizal Fungi**

Thomas Michael Jones

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Department of Biological Sciences

University of Essex

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## **Abstract**

The Arbuscular Mycorrhizal (AM) fungi are a group of obligate plant root endosymbionts, which form associations with an estimated two thirds of terrestrial plant species. Their extra-radical mycelium extends throughout the soil and absorbs nutrients that are transferred to their host plant in exchange for a purely phytogenic carbon supply. Due to their ubiquity and their functional importance, they are the subject of much research into their community ecology, yet much is still unknown. For instance: whether or not AM fungi display preference for certain host plant species; how environmental heterogeneity and energy availability affect communities; and the relative influence of niche and neutral processes. This thesis describes experiments which profile AM fungal communities and environmental properties of their habitat at different spatial scales in different plant species. Network analysis revealed patterns indicative of niche-based processes structuring AM fungal communities more than neutral processes, phenotypic trade-offs between AM fungi, and of priority effects influencing diversity and unevenness. Difference between plant species in the heterogeneity of surrounding soil was dependent on spatial scale. The effect of decreased carbon allocation on AM fungal communities is greater in more heterogeneous habitats. These results suggest that the detection of host plant preference in AM fungi is dependent on spatial scale of sampling, driven by interspecific variation in plant root architecture, soil physical properties and AM fungal vital rates.

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**Author's Declaration**

I, Thomas Michael Jones, hereby certify that all the work contained within this thesis is the result of my own work and was written by myself.

T. M. Jones

University of Essex

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## List of Abbreviations and Units

Ac: *Agrostis capillaris*

AM fungi: Arbuscular Mycorrhizal fungi

Ao: *Anthoxanthum odoratum*

bp: Base Pairs

Cj: *Centaurea jacea*

D1: Depth 1

D4: Depth 4

DF: Decreasing Fill

Fr: *Festuca rubra*

HH: High Heterogeneity

HI: *Holcus lanatus*

HL: High Light intensity

IH: Intermediate Heterogeneity

K(r): Ripley's K function

Kmm(r): Mark correlation function

lat: Latitude

LH: Low Heterogeneity

LL: Low Light intensity

lon: Longitude

Lv: *Leucanthemum vulgare*

ML: Medium Light intensity

NMDS: Nonmetric Multidimensional Scaling

NODF: Nestedness metric based on Overlap and Decreasing Fill

OTU: Operational Taxonomic Unit

PI: *Plantago lanceolata*

PO: Paired Overlap

Ra: *Rumex acetosa*

TRFLP: Terminal restriction fragment length polymorphism

WNODF: Weighted Nestedness based on Overlap and Decreasing Fill

# **Chapter 1: Introduction**

## **1.1 Biodiversity research**

Many of the patterns observed in biological communities have been known throughout history. However, the study of the mechanisms and processes underlying these patterns, such as the positive correlation between species richness and area, known as the species-area relationship (Preston, 1960), is relatively recent. Over the last half-century, considerable progress has been made in the study of ecology at the level of whole communities (Morin and Morin, 1999), to the benefit of the understanding of the complexity of biological systems. For instance, the concept of different interdependent levels of species diversity; the idea that the total species diversity in a landscape, or gamma ( $\gamma$ ) diversity, is determined by the mean species diversity at sites or samples on a local scale (alpha ( $\alpha$ ) diversity) and the differentiation among those sites (beta ( $\beta$ ) diversity) (Whittaker, 1960) has proved useful in community ecology. This concept has provided a theoretical framework for community ecology subdivided into broad yet biologically meaningful spatial scales. This has allowed experimental testing of the niche theory, which states that species differ in their response to their environmental conditions, and therefore it is the environment that determines their spatial distribution. The introduction of theoretical models to describe species abundance distributions and concepts such as the carrying capacity of ecosystems (Preston, 1962) and the dynamic equilibrium model of island biogeography (MacArthur and Wilson, 1963) has improved the theoretical framework in which studies of community ecology can be based. For instance, island biogeography has provided researchers with both a context and the model systems within which hypotheses about ecology, evolutionary biology and biogeography can

be tested (Whittaker and Fernández-Palacios, 2007). Vital as these developments have been to our understanding of biological systems, the emphasis of biodiversity research has, until recently, been on describing and explaining the factors affecting species coexistence and how these factors regulate the observed diversity. The last twenty years has seen a paradigm shift in the perception of diversity, which has led to the overarching aim of explaining the effects of biodiversity on ecosystem functioning (Hillebrand and Matthiessen, 2009). This shift in emphasis has revealed the importance of biodiversity in maintaining ecosystem process rates and thus the complexity and stability of communities over time (Hooper *et al.*, 2005). Given the fundamental importance of biodiversity in ecosystem functioning, understanding the mechanisms and processes underlying the patterns of biodiversity at all spatial scales is vital to the maintenance of resilience to environmental change in biological communities.

## **1.2 Community ecology of microbes**

Despite such ideological advances, effective study of the community ecology of certain groups of organisms has remained a complicated pursuit throughout much of history. The processes and mechanisms structuring communities of microorganisms, for instance, have often proved difficult to resolve, despite their role as a functionally and numerically significant portion of all terrestrial ecosystems (Whitman *et al.*, 1998). Their propensity for various, complex methods of reproduction, poor amenability to culture and often a lack of obvious, discrete individuals and clearly defined morphological differences between species confound attempts to resolve the community ecology of these organisms (Jeffries, 1997). These difficulties were manifest in the fundamental disagreement between early microbial ecology pioneers over the relative influence of certain mechanisms on microbial communities. In 1838,

German naturalist Christian Ehrenberg concluded that, as a result of infusion experiments involving micro-organisms, community composition depended largely on chance. Martinus Beijerinck, in 1913, suggested that it was, in fact, the environmental conditions which determined the presence or absence of any particular species of microorganism. This led to Baas Becking's (1934) famous statement that "Everything is everywhere: but the environment selects", which linked deterministic and stochastic processes in their influence on microbial communities, by its assumption that stochastic processes, such as dispersal limitation, result in the ubiquity of microbes, but deterministic processes, such as environmental filtering, act at more local scales to determine the structure and composition of microbial communities. Broadly, stochastic components of community dynamics are those that are unpredictable, whereas deterministic components are predictable (Lande *et al.*, 2003). Therefore stochastic components are indicative of neutral processes influencing community assembly and deterministic components are indicative of niche-based processes. The use of modern molecular techniques is, however, beginning to overcome some of these limitations. Indeed, methods and theory previously reserved for the study of macro-organisms are now regularly applied to studies of Microbial communities (Horner-Devine *et al.*, 2007, Manter *et al.*, 2010, Fierer *et al.*, 2012).

### **1.3 High throughput and next generation molecular techniques**

Since the 1970s, the technological advances in methods of profiling natural communities have been rapid. The rise of molecular techniques can be said to have started with Kleppe *et al.*, (1971), who developed a technique for repair replication of short synthetic DNA templates. They used DNA polymerase enzymes from a range of sources (including *Escherichia coli*) and short strands of nucleic acids, or primers,

to act as starting points for the replication process. A similar, primer-mediated technique, called polymerase chain reaction (PCR), was subsequently developed that can amplify specific target DNA sequences, resulting in the exponential increase in the number of copies of the original sequence (Saiki *et al.*, 1985, Mullis *et al.*, 1986). This technique eventually revolutionised the study of microbes, allowing DNA to be studied with considerably more accuracy and ease. In 1976, Chien *et al.* isolated and purified a DNA polymerase with a temperature optimum of 80°C, from the thermophilic bacterium *Thermus aquaticus*. The use of this 'Taq DNA polymerase' enabled the specific amplification of DNA to take place at higher temperatures, which improved the specificity and yield of the PCR (Saiki *et al.*, 1988). The development of molecular cloning techniques which used restriction endonucleases and DNA ligases to specifically cleave DNA and recombine it with that of a suitable cloning vector such as *E.coli* also allowed the replication of specific DNA sequences (Jackson *et al.*, 1972). Around the same time, the improvement of methods for determining the sequence of nucleotides in nucleic acids greatly facilitated the study of the DNA sequences that could now be replicated with such accuracy and speed (Sanger and Coulson, 1975, Maxam and Gilbert, 1977). Since then, considerable progress has been made in sequencing technology, including the development of such high-throughput, Next-Generation sequencing (NGS) technologies as 454 pyrosequencing and Illumina dye sequencing. 454 pyrosequencing involves many picolitre-volume wells, in each of which is a DNA fragment attached to a primer coated bead in a droplet of emulsion consisting of pyrophosphates. Solutions of the different nucleotides are sequentially added and removed from the wells, and ATP sulfurylase and luciferase are used to generate light when the pyrophosphates are freed as the DNA extends. Light is generated



only when the nucleotide in solution complements the first unpaired base of the template DNA (Margulies *et al.*, 2005). The detection of the light generated and the relative intensity of each signal allow determination of the DNA nucleotide sequence. Illumina dye sequencing operates on a similar basis, although instead of primer-coated beads in wells, DNA fragments from the community of interest are applied to a flow cell. Through a process of bridge amplification, millions of clusters, each containing around 1000 clonal amplicons, are created on the flow cell. Then a “sequencing by synthesis” approach takes place, during which fluorescently labelled deoxynucleotides are applied to the flow cell in turn. After each round of synthesis, the clusters are excited by a laser which causes a fluorescent signal characteristic of the most recently added deoxynucleotide. This fluorescent signal is detected by a CCD camera and the sequence of millions of DNA fragments can be recorded simultaneously (Glenn, 2011). However, until very recently, NGS has been cost prohibitive and many other high-throughput approaches have been utilised. Terminal restriction fragment length polymorphism (TRFLP) analysis is a quantitative molecular technique used to profile microbial communities based on the lengths of the restriction fragments of DNA amplified using fluorescent primers (Liu *et al.*, 1997). When used in conjunction with a clone library, it is a useful tool for determining the diversity, composition and structure of microbial communities, as it produces an accurate, reproducible characterization of the community, the composition of which can be revealed by the clone library (Clement *et al.*, 1998, Vandenkoornhuyse *et al.*, 2003). As the benefits associated with faster, cheaper and more effective methods of detecting and sequencing DNA are manifold and span numerous scientific disciplines, the progress of this technology is unlikely to slow down in the near future. Indeed, combining the latest molecular techniques with

current ecological theory is the next big challenge and will inevitably yield novel insights into important questions in microbial ecology.

#### **1.4 Arbuscular mycorrhizal fungi**

Such technological and ideological advancements have contributed significantly to our knowledge of the community ecology of a wide range of organisms. The arbuscular mycorrhizal (AM) fungi are one such group of organisms with considerable functional significance (Rosendahl and Matzen, 2008). AM fungi belong to the phylum Glomeromycota (Schüßler *et al.*, 2001) and are the most common mycorrhizal fungi, ubiquitous in natural ecosystems (Smith and Read, 2008), forming associations with an estimated two-thirds of plant species (Helgason and Fitter, 2009). They are obligate plant-root endosymbionts which obtain 100% of their carbon from their host plant, via the intraradical mycelium (Helgason and Fitter, 2009), and confer numerous beneficial effects on their host plants, including enhanced N uptake (Hodge *et al.*, 2001, Leigh *et al.*, 2009), enhanced P uptake, increased protection from fungal pathogens, greater drought resistance (Newsham *et al.*, 1995, Helgason *et al.*, 2007), increased uptake of micronutrients such as zinc and copper (Marschner and Dell, 1994) and decreased restriction of plant growth in soils with elevated levels of Arsenic (Leung *et al.*, 2010), Lead (Ma *et al.*, 2006) and Lanthanum (Chen and Zhao, 2009), to name a few.

#### **1.5 Community ecology of AM fungi**

The Mycorrhizal-plant mutualism is the most widespread diffuse mutualism (a mutualism whose component species can interact with multiple partners (Gove *et al.*, 2007)) currently known (Smith and Read, 2008). Due to their ubiquity and their functional importance in terrestrial ecosystems worldwide, they are the subject of

much research into their community ecology. As a result, much has already been revealed about how they interact with their environment. For example, there is evidence that the diversity (number of species present), composition (identity of species present) and structure (relative abundances of species present) of AM fungal communities can determine the diversity, composition and structure of associated plant communities (van der Heijden *et al.*, 1998). Only recently, however, has Beta ( $\beta$ ) diversity (Whittaker, 1960) of AM fungal communities been incorporated into the study of their spatiotemporal dynamics (Dumbrell *et al.*, 2010b). Given that the global metacommunity of any species is determined by the number of and difference between many local communities (Etienne *et al.*, 2007), this recent incorporation seems surprising. The heterogeneous distribution of individuals, typical of soil organisms like AM fungi, means that dispersal may often be very limited, especially among those taxa that are absent from the uppermost soil layers. This characteristic “spatial patchiness” (Fitter, 2005) of AM fungal communities is almost certainly a result of both deterministic and stochastic processes, as dispersal rates will limit the spread of AM fungi on smaller scales, and deterministic processes will affect their distribution on larger scales. Dispersal could be interpreted as a deterministic process, as spore size, soil movement by animal dispersal agents and growth rates and architecture of colonised roots and hyphae are potentially predictable (Sylvia and Will, 1988). However, at least in the case of root and hyphal architecture and growth, there is undoubtedly a stochastic element involved (Fitter *et al.*, 1991). The effects of atmospheric change (Cotton *et al.*, 2015) and neighbouring plant identity (Mummey *et al.*, 2005a) on AM fungal community composition have been revealed largely due to technological advances in community profiling. Cotton *et al.* (2014) demonstrated that TRFLP analysis can be used quantitatively to profile

AM fungal communities, as no PCR bias was detected. Therefore the relative abundances of AM fungal taxa within samples can be interpreted as an accurate reflection of the relative abundances of those taxa in the original sample before PCR amplification. The capacity for other PCR-based high throughput/ Next-generation sequencing (NGS) methods to reliably and accurately profile AM fungal communities is evident. Such molecular methods have been put to effective use in investigating AM fungal ecology. Indeed, the lack of PCR bias in AM fungi means that all PCR-based methods are robust and reliable, and as such, they are the ideal model organism for addressing broad questions in community ecology. Further insight into how their plant hosts affect the composition of AM fungal communities throughout the growing season has been gained using NGS (Dumbrell *et al.*, 2011), as well as the influence of stochastic processes in community assembly at certain spatial scales (Lekberg *et al.*, 2011). Despite these many advances, much is still unknown about the community ecology of AM fungi.

### **1.6 Spatial scale-dependence of host plant preference**

In contrast to ectomycorrhizal fungi, there are no cases of absolute host specificity in AM fungi (Bidartondo *et al.*, 2002). Host plant preference in AM fungi, however (non-random associations between AM fungi and host plant species), is a much more controversial issue, and has been detected only in a subset of all the studies into AM fungi-host plant dynamics. Various types of host preference have been recorded. Lekberg *et al.*, (2011) reported that the distribution of two plant species that are rarely and poorly colonised by AM fungi (*Dianthus deltoides* and *Carex arenaria*) correlated significantly with AM fungal community composition. They suggest that this provides evidence for “host quality” playing a role in the structuring of AM fungal communities. Helgason *et al.* (1998) recorded distinct communities of AM fungi from

woodland soils and arable soils, although this could be a result of differing degrees of disturbance in agriculture and woodland soils driving compositional divergence between the two habitats. Similarly, Öpik *et al.* (2009) suggested that host preference occurred at the level of ecological groups of both plant and fungal partners, such that specialist AM fungal taxa are more likely to associate with habitat specialist plant species, and generalist AM fungi more likely to associate with generalist plant species. This could be a co-occurrence caused by the fungi and the host plants having a similar range of environmental variables which they can tolerate. Host plant preference has been recorded between coexisting grass and forb species (Vandenkoornhuyse *et al.*, 2002b) and between coexisting grass species (Vandenkoornhuyse *et al.*, 2003). Host plant preference has even been reported to vary between particular genotypes from the same AM fungal species (Croll *et al.*, 2008) and distinct AM fungal communities have been recorded in different cultivars of the same host plant species (Mao *et al.*, 2014). This is suggestive of the action of environmental variables other than host plant identity in structuring AM fungal communities. Merryweather and Fitter (1998) reported that AM fungi inside roots and their spores in the soil significantly correlated with the dominant plant species in forest communities. For example, *Glomus* was more abundant under a canopy of sycamore, while *Acaulospora* was more abundant under oak. Similarly, Mummey *et al.*, (2005b) reported a significant influence of the presence of a neighbouring invasive plant species, *Centaurea maculosa*, on the AM fungal communities associated with the grass *Dactylis glomerata*. Many of these results could be caused by other environmental factors associated with the soil environment around certain plant species. Other studies, on the other hand, detected no host specificity among AM fungi (Klironomos, 2000, Santos *et al.*, 2006). Some researchers relate this

apparent host plant preference to environmental conditions and dispersal constraints (Isobe *et al.*, 2011, Torrecillas *et al.*, 2013), and have found distinct AM fungal communities in geographically distant individuals of the same plant species (Isobe *et al.*, 2011). Dumbrell *et al.* (2008) demonstrated the scale-dependence of estimating diversity in a tropical system, reporting a difference in the community response to disturbance at different spatial scales. Differences between habitats in biotic and abiotic factors could, therefore, influence both the plant and fungal communities, resulting in the perception of an apparent host plant preference in AM fungi. These differences between habitats could be dependent on spatial scale. The lack of consensus regarding host plant preference among AM fungi could, therefore, also be due to differences in the spatial scales of studies. Indeed, it has been observed that in maize (*Zea mays*), only at spatial scales greater than 1 metre does the AM fungal community composition differ between plants. Understanding how AM fungal host plant preference depends on the spatial scale of sampling is an important part of their community ecology about which we still know very little.

### **1.7 Niche and neutral theories**

While there is evidence that both niche and neutral processes structure microbial communities, the relative importance of each remains a contentious issue (Holt, 2006)(Holt, 2006)(Holt, 2006)(Holt, 2006)(Holt, 2006). It is likely that while stochastic processes are more influential at smaller spatiotemporal scales, deterministic processes operate more at larger scales (Ferrenberg *et al.*, 2013, Dini-Andreote *et al.*, 2015). Using Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis, Dumbrell *et al.*, (2010b) found that AM fungal species abundances in communities taken from a grassland, wood and heath ecotone in the north of England fitted a zero-sum multinomial distribution. Given that the zero-sum

assumption of neutral theory assumes that environmental constraints on communities are constant and that the number of individuals within a community are never fewer than these environmental constraints permit, the zero-sum multinomial species abundance distribution indicates that any difference in species composition between communities is a result of distance-decay driven by neutral processes such as dispersal limitation, and not of niche-based processes. They also reported evidence for a spatial structure in the community suggestive of the influence of dispersal limitation, another key neutral process. Both of these are indicative of neutral processes structuring the community. However, deterministic processes were more important in regulating the composition of the AM fungal community in this study. Specifically, soil pH, C/N ratio and phosphorus content were the main factors that determined the structure and composition of the community. Lekberg *et al.*, (2011) found, contrary to their own hypothesis that severe disturbance events would shift the AM fungal community composition towards disturbance-tolerant species, that the reassembly of the community was unpredictable, and therefore dominated by stochastic, as opposed to deterministic, processes. Fitting empirical data to species abundance distributions can be useful in objectively comparing different assemblages, and biological explanations can often be assigned based on which species abundance distribution (SAD) best fits the community. Dumbrell *et al.*, (2010a) observed species abundance distributions that fitted both lognormal and broken-stick models in a woodland/grassland AM fungal community and in 32 previously published datasets, suggesting a probabilistic division of niche space among the species in these communities, as predicted by neutral theory. Both of these models, however, failed to predict the idiosyncratic overdominance of the most dominant species observed in the community, with one species accounting for up to

40% of the total abundance of any community. This overdominance of a single taxon has been observed on multiple occasions (e.g. Helgason *et al.*, 1998, Daniell *et al.*, 2001, Husband *et al.*, 2002, Dumbrell *et al.*, 2010a). Dumbrell *et al.*, (2010a) suggest that this uncommonly observed pattern could be a result of the overarching benefit received by the first species to colonize a previously uncolonised plant root. As AM fungi receive all their carbon from their host plant, and uncolonised plant roots will remain so until they come into physical contact with active fungal mycelia, the first AM fungal species that comes into contact with such a root will receive considerably more carbon than subsequent colonisers. A positive feedback mechanism results, as the fungus can use this extra carbon to extend its extra-radical mycelial network and come into contact with more uncolonised roots, gaining further additional carbon. While the differences in abundance among the majority of species in such a community are minimised by strong interspecific interactions (Poulin *et al.*, 2008), one species may be able to numerically dominate to such an extent as a result of these greater recruitment rates. In the study by Dumbrell *et al* (2010a) the taxonomic identity of the most abundant AM fungal species was unpredictable and idiosyncratic, suggesting that stochastic processes play a significant role in determining which species will numerically dominate any community. While stochastic processes may play a more important role in the identity of the most abundant species, deterministic processes must by no means be discounted altogether. Interspecific differences in vital rates among the AM fungi and their plant hosts, such as relative allocation to spores and mycelium, spore germination rates and growth rates of mycelium and roots may also contribute (Lekberg *et al.*, 2011). Not only can these rates be affected by abiotic factors, but the differences in growth patterns and functions between AM fungal species in natural communities and in



culture are largely unknown (Rosendahl and Stukenbrock, 2004, Sýkorová *et al.*, 2007a). Lower colonisation rates in ectomycorrhizal fungi have been shown to affect the outcome of competition, although the outcome of competition is strongly dependent on the order of colonisation (Kennedy *et al.*, 2009). Hart and Reader (2002) observed that while members of the *Gigasporaceae* displayed relatively slow rates of colonisation of plant roots relative to investment in extraradical mycelium, *Glomeraceae* species, on the other hand, tended to colonise roots more rapidly and amass biomass in the extraradical mycelium at a lower rate. These functional differences among AM fungal taxa could be related to various mechanisms for promoting growth of the host plant, e.g. reduction of infection by pathogens and promotion of growth by enhancing P uptake and improving water relations (Maherali and Klironomos, 2007, Powell *et al.*, 2009). Powell *et al.* (2009) found no evidence for a trade-off in allocation of biomass between root and soil colonization, instead finding evidence for a positive correlation. This suggests further scope for differentiation amongst AM fungal species and therefore interspecific variation in responses to environmental variables. Dumbrell *et al.*, (2011) observed distinct seasonal assemblages of AM fungi in a grassland system, as a result of priority effects caused by a seasonally changing phytogenic carbon supply. Davison *et al.*, (2011) suggest that these seasonal changes in the composition and structure of AM fungal communities could be evidence for a seasonal cycle of niche and neutral processes dominating at different times of year. They found that the distinct AM fungal communities associated with the ecosystem functional types of plants (i.e. generalists or specialists) tended to develop later in the summer and not be so pronounced at the start of spring. Davison *et al.*, (2011) postulate that the colonisation of new plant roots by AM fungi in the Spring is largely stochastic, but

some degree of host plant preference, as a result of deterministic processes, becomes apparent later in the season. Bennett *et al.* (2013) backed up this hypothesis with their observation that the structure of the AM fungal-plant interaction network was dynamic throughout the growing season, providing evidence for switching among partners by AM fungi and plants. Dumbrell *et al.*, (2010a) urge further study into stochastic processes over sufficiently long timescales to determine their importance in structuring AM fungal communities. Indeed it is likely that deterministic processes are more important in structuring AM fungal communities at larger spatial and longer temporal scales. This has been observed to be the case in other microbial systems (Ferrenberg *et al.*, 2013, Dini-Andreote *et al.*, 2015). Determining a method to calculate the relative importance of stochastic and deterministic processes in the structuring of AM fungal communities over various spatial and temporal scales would be useful for land managers and conservationists, for instance, in planning to optimise AM fungal diversity.

### **1.8 Network structure, nestedness and modularity**

Despite its potential for insight into the processes that structure AM fungal communities, the use of network analysis tools in studying AM fungal communities is a relatively recent concept. The nestedness pattern, which is said to exist in a metacommunity where the species assemblages in the most species-poor communities tend to be proper subsets of the more species-rich assemblages, has been observed in AM fungal communities (Verbruggen *et al.*, 2012, Bennett *et al.*, 2013). Very few studies have investigated nestedness in AM fungal communities, and even fewer have used a more informative, quantitative nestedness metric. Given that the degree of nestedness in any metacommunity has implications for the coexistence of species and thus the complexity of communities, this shortcoming is a

considerable one. How the spatial scale and depth of sampling affect conclusions drawn about the nestedness of metacommunities is largely unknown. Similarly, the detection of modules (subgroups in the network within which organisms are more likely to interact with each other than with organisms outside the subgroup) in a network is likely to depend on spatial scale of sampling. Modules within a network representing AM fungal species at different sites indicate endemism in certain species and are therefore a useful way of describing the dispersal ability and rarity of those species. While there are potential limitations of analysing AM fungi-plant interaction networks using nestedness metrics, such as whether or not frequency of occurrence of a particular AM fungal OTU within a certain plant correlates with interaction strength (Caruso *et al.*, 2012), this does not affect the results and conclusions of the current work. This is because the networks analysed are occurrence matrices, not interaction matrices, and no attempt to link frequency of occurrence with strength of interaction is made.

## **1.9 Environmental heterogeneity and energy**

### *1.9.1 Heterogeneity effects on diversity*

Environmental heterogeneity is the complexity and/or variability of a system property in space and/or time. Complexity refers to a qualitative descriptor of a system property, whereas the variability refers to a quantitative descriptor (Li and Reynolds, 1995). Thus it is most often the variability of environmental parameters which is analysed in studies into the effects of environmental heterogeneity. Ettema and Wardle (2002) define heterogeneity specifically as a “patchiness”, or the degree to which a distribution of a system property displays positive spatial autocorrelation (aggregation). The habitat-heterogeneity hypothesis proposes that a high degree of

spatial heterogeneity promotes the persistence of a high degree of biological diversity, in terms of species richness (McIntosh, 1986). This is because in more complex habitats, the limiting resources can be more readily subdivided, which can lead to greater specialisation, and therefore the co-existence of a greater number of species (Pianka, 1966). Habitat differentiation among different species, in the form of specialization in the range of particular environmental parameters in which a species can exist, for instance, is evidence in support of this explanation (Silvertown and Wilkin, 1983). Indeed, positive relationships between species richness and rate of diversification have been observed in certain macro-organisms such as plants and arthropods (Emerson and Kolm, 2005). However, the effect of environmental heterogeneity on species diversity is varied, with studies reporting positive (Lundholm and Larson, 2003), neutral (Reynolds *et al.*, 2007) and even negative (Cramer and Willig, 2005) effects. In fact, negative heterogeneity-diversity relationships are now known to be more common than once thought, especially at smaller spatial scales (Laanisto *et al.*, 2013). One review found that 83% of observational studies reported a positive correlation between plant species diversity and at least one metric of spatial heterogeneity (Lundholm, 2009). The factors whose spatial heterogeneity significantly positively correlated with diversity included elevation, topography, slope, aspect, temperature, bedrock geology, flooding frequency, land use, light, nitrate, nitrogen and organic matter. The factors whose spatial heterogeneity negatively correlated with diversity were rainfall, soil depth, topography and soil chemistry. Interestingly, positive, negative and unimodal relationships were observed between plant diversity and heterogeneity in topography. Only 27% of studies in which environmental heterogeneity was experimentally controlled reported positive heterogeneity-diversity relationships.

Heterogeneity in soil type, topography and disturbance positively correlated with plant diversity in the experimental studies.

#### *1.9.2 Energy effects on diversity*

The total amount of energy available to the biological components of an ecosystem has a considerable effect on the species diversity of that system (Leigh Jr, 1965). The most frequently observed pattern in productivity-diversity relationships was, for a long time, a hump-shaped relationship, in which intermediate levels of productivity produced the highest diversity (Fukami and Morin, 2003). The apparent ubiquity of such a pattern has since been overturned, and U-shaped patterns, along with positive and negative correlations as well as non-significant relationships have been observed, none of which seem to predominate (Waide *et al.*, 1999). Recent work indicates, however, that in herbaceous grassland communities, the hump-shaped relationship is the most common productivity-diversity relationship (Fraser *et al.*, 2015). The explanation posited for this is that in habitats of low productivity, environmental stresses limit the number of species able to co-exist, and in highly productive habitats, a small number of highly competitive species tend to dominate. While the diversity of a wide range of species has been shown to correlate with variables related to energy availability (Hawkins *et al.*, 2003), therefore, the nature of the relationship depends on taxonomic group and habitat type (Mittelbach *et al.*, 2001). Explanations for variation among the patterns include the influence of disturbance, spatial scale and niche specialization (Fukami and Morin, 2003). While energy-diversity relationships in AM fungal communities are largely unknown, given the tendency for overdominance in AM fungal communities to result in high unevenness and low species-richness (Chapter 3), a hump-shaped shaped pattern is likely.

Very little is known about how environmental heterogeneity and available energy within a system interact to affect the diversity of microbes such as AM fungi. As AM fungi obtain all their carbon from their host plant, determining the effects of energy and heterogeneity on their community structure would provide unprecedented insight into the community ecology of obligate mutualists.

### **1.10 Spatial patterning of the AM fungal microhabitat**

It is clear that AM fungal communities are influenced by a wide range of soil physical properties. However, little is known about the spatial patterns in the variability of these properties, which comprises the environmental heterogeneity to which AM fungal communities are subject. Among the environmental parameters which affect AM fungal communities are biotic factors such as disturbance and competition from other soil biota such as protozoa, nematodes, arthropods and burrowing mammals (Fitter and Garbaye, 1994) and the chemical influence of root exudates such as strigolactones (Besserer *et al.*, 2006), along with the effects of host plant identity (Davison *et al.*, 2011). Additionally, abiotic factors such as the organic matter content of soil (Joner and Jakobsen, 1995), pH (Dumbrell *et al.*, 2010b), soil nutrient status (Fitzsimons *et al.*, 2008) and disturbance (Souza *et al.*, 2005, IJdo *et al.*, 2010) can contribute to the structure, composition and diversity of AM fungal communities. While the environmental factors that affect AM fungal communities have been studied extensively at the landscape scale (Öpik *et al.*, 2006, Hazard *et al.*, 2013), little is known about how their habitat varies at small scales. It is likely that the heterogeneity of many of these properties in natural AM fungal habitats differs between host plants and spatial scales, potentially contributing to the non-random assemblages of AM fungi in different host plants.

## **1.11 Thesis aims and structure**

### *1.11.1 Thesis rationale*

AM fungi are the most widespread diffuse mutualism known, and the structure of their communities has implications for plant diversity, crop nutrition, carbon sequestration and soil health. There is a lack of consensus about whether or not AM fungi display host plant preference. Variation in the degree of heterogeneity of the soil environment at different spatial scales may be driving the perceived non-random assemblages of AM fungi among different host plant species. Determining the influence of spatial scale on AM fungal community structure, diversity and composition will potentially inform conservation and management practice, as well as future research into their ecology. Analysis of network patterns in AM fungal metacommunities can reveal information about the process of root colonisation and its effect on community structure, about phenotypic trade-offs between species, about the relative influence of stochastic and deterministic processes, and about the resilience of metacommunities against extinction events. The role of energy availability and environmental heterogeneity, both separately and together, is poorly resolved in soil microbes, and especially in AM fungi. Root colonisation and AM fungal biomass both in the host plant and in the soil are highly dependent upon the availability of energy. As such, the manner in which heterogeneity affects carbon assimilation in AM fungi has major implications for carbon cycling and sequestration. Such research into the effects of energy availability and environmental heterogeneity on AM fungal communities has greater relevance as natural and anthropogenic change to the environment increases.

### *1.11.2 Thesis objectives*

Objective 1 – chapter 2: **Spatial patterns of arbuscular mycorrhizal fungal communities as a potential driver of perceived host-plant preference:** To determine whether the degree of spatial heterogeneity perceived by AM fungi differs between plant species, and the manner in which these differences in heterogeneity affect how AM fungal community diversity and composition scale through space. A biodiversity experiment set up in Wageningen University and Research Centre is used to investigate the spatial scaling properties of AM fungal communities in four host plant species.

Objective 2 - chapter 3: **Spatial dependence of arbuscular mycorrhizal fungal network properties:** To determine the relative influence of stochastic and deterministic processes, whether network properties are dependent on spatial scale of sampling, and to test for evidence of phenotypic trade-off and priority effects in AM fungi. AM fungal metacommunities from the Wageningen Biodiversity experiment are profiled using 454-pyrosequencing and their nestedness and dominance structure analysed.

Objective 3 - chapter 4: **Spatial patterning of the soil environment and its effect on natural AM fungal communities:** To quantify the degree of spatial heterogeneity within the AM fungal habitat, and to determine whether the difference in spatial heterogeneity between host plant species could contribute to the detection of host plant preference. Soil environmental parameters are measured surrounding two host plant species at a range of spatial scales in a natural plant community.

Objective 4 - chapter 5: **Effects of environmental heterogeneity and energy availability on arbuscular mycorrhizal assemblages:** To test the effects of energy



and heterogeneity on root colonisation by AM fungi, and physical properties of host plants, and to predict the effects on AM fungal community diversity, structure and composition. A plant growth experiment with soil inoculum from a grassland ecosystem is used to manipulate the available energy and environmental heterogeneity to which AM fungal communities are subject.

## **Chapter 2: Spatial patterns of arbuscular mycorrhizal fungal communities as a potential driver of perceived host-plant preference**

### **2.1 Summary**

- The spatial scale of sampling can affect conclusions made about the underlying mechanisms structuring the study community, so the manner in which ecologically important taxa scale through space is of considerable importance.
- A lack of consensus exists about whether or not AM fungi exhibit host plant preference. One potential cause for this is variation in the spatial scale of studies.
- A ten-year biodiversity experiment was used to investigate the aboveground (plant) populations (two grasses and two forbs) and the belowground (AM fungal) communities and the correlations between them across spatial scales.
- The grasses displayed less heterogeneity than the forbs in their aboveground spatial patterns, but higher AM fungal species richness and Beta diversity
- Differences in the spatial scaling of AM fungal communities associated with the different host plant species render the detection of host plant specificity highly dependent on spatial scale of sampling.

### **2.2 Introduction**

Understanding mechanisms and processes structuring natural communities is a central goal in ecology. The biodiversity of these communities is a major driver of ecosystem functioning, which in turn influences the ecosystem services they provide (Cardinale *et al.*, 2012). Yet, despite being widely studied, in many important

biological systems, such as microorganisms, much of this basic ecology remains poorly understood (Green *et al.*, 2004). Given the vital role of micro-organisms in the functioning of all natural ecosystems (Prosser, 2002), this shortcoming seems considerable.

It has long been assumed that microorganisms have such a high degree of vagility afforded to them by their small size and high abundance that no effect of dispersal limitation exists, and that their local diversity, driven by environmental parameters, varies little around the world (Bass-Becking, 1934). However, dispersal limitation and distinct spatial patterns have been reported in microbial communities (Husband *et al.*, 2002, Green *et al.*, 2004). Spatial patterns that are species-specific or even idiosyncratic may yield results, and therefore conclusions about the study organism, that are highly scale-dependent (Robeson *et al.*, 2011). Contrary to Bass-Becking's (1934) hypothesis that "everything is everywhere, but, the environment selects", microbes display spatial patterns in their occurrence and abundance due in part to dispersal limitation (Peay *et al.*, 2010) and in part due to environmental heterogeneity (Ramette and Tiedje, 2007). The environmental heterogeneity to which microbes are subject and by which they are affected is dependent upon the study taxon and the environment from which they are sampled. Soil microbes associated with plant roots, e.g. arbuscular mycorrhizal (AM) fungi (Dumbrell *et al.*, 2010b, Lekberg *et al.*, 2011), can be affected by soil physical and chemical properties (Hazard *et al.*, 2013) and by the distribution of roots of the host plant (Husband *et al.*, 2002).

The AM fungi are a phylum (Glomeromycota) of globally distributed, obligate plant root endosymbionts which provide their plant hosts with a range of benefits, including improved nutrient status, drought tolerance and pathogen resistance

(Smith and Read, 2008). The AM-plant relationship is the most widespread plant symbiosis known (Simon *et al.*, 1993); AM fungi are present in around 80% of all known vascular plant species (Schüßler *et al.*, 2001), including many ecologically and economically important species. AM fungi both affect (van der Heijden *et al.*, 1998, Klironomos, 2003) and are affected by (Bever *et al.*, 1996) the structure of their host plant communities. Host plant preference by AM fungi (non-random associations between AM fungi and host plant species) has been detected only in a subset of all the studies into AM fungi-host plant dynamics, and various types of host preference have been recorded. Helgason *et al.* (1998) recorded distinct communities of AM fungi from woodland soils and arable soils. Similarly, Öpik *et al.* (2009) suggested that host preference occurred at the level of ecological groups of both plant and fungal partners, such that specialist AM fungal taxa are more likely to associate with habitat specialist plant species, and generalist AM fungi more likely to associate with generalist plant species. Host plant preference has been recorded between coexisting grass and forb species (Vandenkoornhuyse *et al.*, 2002b) and between coexisting grass species (Vandenkoornhuyse *et al.*, 2003). Host plant preference has even been reported to vary between particular genotypes from the same AM fungal species (Croll *et al.*, 2008) and distinct AM fungal communities have been recorded in different cultivars of the same host plant species (Mao *et al.*, 2014). Other studies relate this apparent host plant preference to environmental conditions and dispersal constraints (Isobe *et al.*, 2011, Torrecillas *et al.*, 2013), and have found distinct AM fungal communities in geographically distant individuals of the same plant species (Isobe *et al.*, 2011). Dumbrell *et al.* (2008) demonstrated the scale-dependence of estimating diversity in a tropical system, reporting a difference in the community response to disturbance at different spatial scales. The lack of

consensus regarding host plant preference among AM fungi could also be due to differences in the spatial scales of studies.

One principle in ecology that has proved consistent across systems since its initial discovery is that the total number of species increases as the area sampled increases (De Candolle, 1855, Arrhenius, 1921). This is now known as the Species-Area Relationship (SAR), and was perhaps the first ecological relationship to be recognised. So widely documented is this relationship that it is often considered a true universal ecological law (Preston, 1948, Simberloff, 1974, MacArthur and Wilson, 2001). It states that:

$$S=cA^z$$

*equation 1*

where S is the number of species in area A. The intercept in log-log space c and exponent z are constants derived from experimental data, depending on the sampling location and the taxonomic group of study (Preston, 1960). As the area sampled increases, environmental heterogeneity often increases, as a greater degree of complexity and variability of system properties is necessarily encountered in a larger area. This area-dependent increase in heterogeneity, along with niche differentiation amongst species, is perhaps the most common explanation for species-area relationships (Johnson and Simberloff, 1974, Rosenzweig, 1995). A high degree of environmental heterogeneity promotes the persistence of a high degree of biological diversity, in terms of species richness (Ruggiero & Kitzberger, 2004) because in more complex habitats, the limiting resources can be more readily subdivided, which can lead to greater specialisation, and therefore the co-existence of a greater number of species (Pianka, 1966). Therefore, the rate of accumulation

of species as the area sampled increases is greater in more heterogeneous habitats. Thus the exponent  $z$  in equation 1, which indicates the rate of turnover of species across space, and determines the slope of the curve, is heavily dependent upon the degree of environmental heterogeneity in the study area. If the SARs associated with different AM fungal communities intersect at any point over the spatial scales encompassed in the study, then the perceived community diversity is dependent on spatial scale. Habitats with different levels of heterogeneity are therefore likely to host communities with different SARs, potentially producing scale dependencies in estimates of species richness and diversity.

If the spatial patterning of the associated host plants (a) contributes significantly to the degree of spatial heterogeneity as perceived by AM fungi, and (b) differs between host plants, then the rates of accumulation of AM fungal taxa across plots containing different host plants would vary. Therefore estimates of AM fungal diversity for each host plant would be dependent on the spatial scale at which the community is sampled. The advantage of experimental studies over correlational studies in elucidating the processes structuring these communities is evident. Indeed, that the influence of spatial scaling relationships in structuring AM fungal communities remains largely unknown, even considering their importance in ecosystem functioning, represents a considerable gap in current ecological knowledge.

### **2.3 Aims and Hypotheses**

In this study the following hypotheses are being tested:

1. Plant species vary in the degree of environmental heterogeneity perceived by AM fungi

2. Different plant species host different AM fungal communities, only observable at certain spatial scales
3. The perceived environmental heterogeneity affects how AM fungal community diversity and composition scale through space
4. Observed host-plant preference is spatial-scale dependent

Aims:

1. To determine whether the degree of spatial heterogeneity perceived by AM fungi differs between plant species
2. To determine the manner in which plant species-specific differences in heterogeneity affect how AM fungal community diversity and composition scale through space

Differences in AM fungal community diversity, structure and composition are more likely to be detected at the larger spatial scales of this study. Given the high degree of spatial variability in AM fungal communities (Sylvia and Will, 1988, Merryweather and Fitter, 1998, Cheeke *et al.*, 2015) and their dependence on the presence of plant roots, the effects of the scale dependence are more likely to be detected at scales at which the disparity in spatial patterning between host plant species is greater. The environmental heterogeneity to which AM fungal communities are subject is expected to differ between host plant species due to differing spatial patterns between plant species. This difference is expected to influence the AM fungal species accumulation in host plant species, such that the SARs of associated AM fungal communities will intersect within the spatial scales of this study. This will render the observed patterns of diversity dependent on spatial scale of sampling.

Similarly, heterogeneity is expected to be positively correlated with AM fungal species richness and species accumulation across each plot.

## **2.4 Materials and methods**

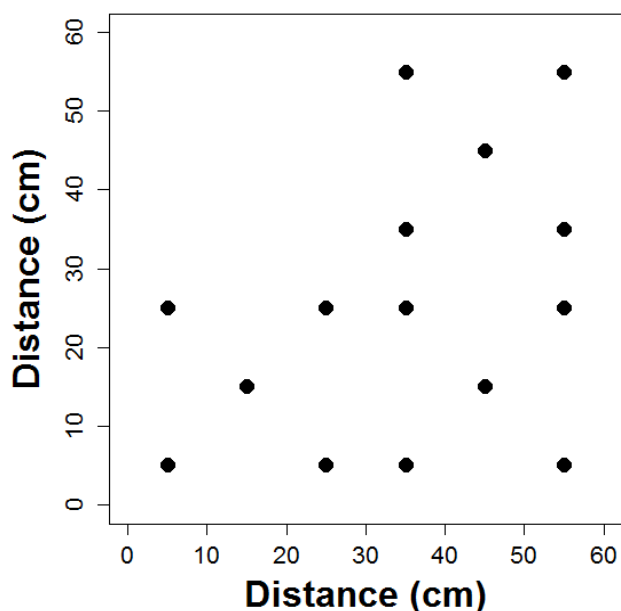
### *2.4.1 Study site*

In order to quantify spatial patterns of both host plant and AM fungal communities, samples from an existing biodiversity experiment that was set up in 2000 were studied (Van Ruijven and Berendse, 2003, Van Ruijven and Berendse, 2009). A description of the biodiversity experiment follows:

108 experimental plots of 1m<sup>2</sup> were established on an arable field in the Netherlands in early spring 2000. Distance between plots was one metre, and blocks were two metres apart. The topsoil in each plot was removed to a depth of 45 cm, exposing the mineral sand layer below the arable soil. Wooden frames measuring 1 × 1 × 0.5 m (l × w × d) were placed into each hole and filled with a mix of arable soil and pure sand (1:3). Seeds were sown on the same mixture and germinated in plug trays in the greenhouse. After three weeks, the seedlings were planted in the plots according to a substitutive design, in which the total seedling density was identical for each plot (144 seedlings per plot). During the first three months, plots were watered regularly to prevent desiccation of the seedlings. After this, no further water was applied. Each year, in early August, the plants were clipped to 2.5 cm above the soil surface and the cut plant material used in a different study. Species were selected from a pool of four grass species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., *Holcus lanatus* L.) and four dicotyledonous species (*Centaurea jacea* L., *Leucanthemum vulgare* Lam., *Plantago lanceolata* L., *Rumex acetosa* L.). Nomenclature follows Van der Meijden (Van der Meijden *et al.*, 1990). All species



are C<sub>3</sub> perennials and commonly coexist on temperate European hay meadows. Species will hereafter be referred to by their genus names. Each block contained monocultures of all species, four mixtures of two and four species, and an eight species mixture. The composition was maintained throughout the experiment by removing seedlings of all other species at monthly intervals during each growing season. To avoid confounding edge effects, plots were divided into a centre of 60 × 60 cm and a surrounding edge. Only data from the centres were used for the analysis. In the current study, three replicate monoculture plots for each of the four plant species *Festuca*, *Anthoxanthum*, *Centaurea* and *Leucanthemum* were used. Fifteen soil cores (18 mm diameter, 0 – 150 mm depth) were taken from each monoculture in the summer of 2011 in a spatially explicit manner to provide a suite of spatial scales that ranged from the small (between cores in an individual plot) to the



large (between plots of different host plant species) (Fig. 2.1), totalling 180 samples (45 for each plant species). In addition, an aerial photograph was taken in the same place over each plot. Seven wooden stakes (three equally spaced on two sides of the 60 x 60 cm square and one in the centre) were used to ground-truth the scale of the plots.

Figure 2.1. Experimental design of the centre 60 x 60 cm area inside a single plot. Black circles are soil cores taken from the plot for molecular analysis of host plant roots.

## **2.4.2 Molecular Methods**

### *2.4.2.1 DNA Extraction*

Roots from each core were washed free of soil, dried at 70°C for 72 hours and stored until use. 50 mg of root from each soil core was separately homogenised for two minutes in a bead tube containing 4 stainless steel beads. Total community DNA (Plant DNA and AMF DNA) was extracted from the ground roots in each soil core using a PowerPlant DNA isolation kit following the manufacturer's instructions (Mo Bio Laboratories Inc., Carlsbad, CA, USA).

### *2.4.2.2 DNA Amplification and Purification*

A 550 bp partial fragment of the AM fungal Small Subunit (SSU) ribosomal RNA gene was amplified using the universal eukaryotic primer NS31 (Simon *et al.*, 1992) and the primer AM1 which amplifies the major Glomeromycotan families (Helgason *et al.*, 1998). PCR reactions were carried out in the presence of 2mM dNTPs, 0.2 µM of each primer and the manufacturer's reaction buffer in 25 µl reactions (PCR conditions: 95 °C for 2 min; 30 cycles at 94 °C for 0.5 min; 58 °C for 0.5 min and 72 °C for 1 min; and 72 °C for 10 min) on an Eppendorf Mastercycler® personal (Eppendorf Hamburg, Germany). The PCR products were characterised using gel electrophoresis on a 1% agarose gel stained with ethidium bromide and visualised under UV light. To remove humic-acid-based PCR inhibitors, 0.125 µL of T4 gene 32 protein (Roche Diagnostics Ltd, W. Sussex, UK) was added to all PCR reactions. PCR products were purified using QIAquick PCR Purification Kit (Qiagen Ltd, W Sussex, UK).

### *2.4.2.3 Cloning and Sequencing*

In order to produce an AM fungal clone library for each of the host plant species, purified PCR products pooled according to host plant species, then were ligated into pGEM-T Easy vector (Promega Co., Madison, WI, USA) and transformed into *Escherichia coli* competent cells (JM109) (Promega Co., Madison, WI, USA). Putative positive transformants were screened using SP6-T7 amplification and purified and sequenced with the T7 universal primer by GATC Biotech (Konstanz, Germany). BigDye Terminator v3.1 on ABI 3730xl. (sequencing conducted under BigDye™ terminator cycling conditions and run using an ABI3730xl automatic sequencer (Applied Biosystems, Waltham, Massachusetts, USA.).

#### 2.4.2.4 TRFLP Analysis

The 550 bp partial fragment of the AM fungal Small Subunit (SSU) ribosomal RNA gene was amplified from each of the 180 samples of extracted DNA (45 from each plant species) using MyTaq™ Red Mix (Bioline, London, UK) and the aforementioned primers NS31 and AM1, labelled with the fluorescent markers HEX and 6-FAM respectively on the 5' end. PCR was carried out in the presence of 2mM DNTPs, 0.2 µM of each primer, 1 µl of DNA template and 0.125 µL of T4 gene 32 protein in a 25 µl volume reaction (PCR conditions: 95 °C for 2 min; 30 cycles at 94 °C for 0.5 min; 58 °C for 0.5 min and 72 °C for 1 min; and 72 °C for 10 min). Labelled PCR products were purified using QIAquick PCR Purification Kit (Qiagen). Fluorescently labelled PCR amplicons were digested separately with the enzymes Hsp92II and HinfI (10 µl reactions, 0.5 ml enzyme, 0.2 ml bovine serum albumin) and purified using QIAquick PCR Purification Kit (Qiagen). The resulting product was loaded onto an ABI 3730 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) to determine the sizes and quantities of terminal fragments (TFs) in each digest. TRFLP analysis was carried out at the University of Dundee DNA

Sequencing and services (Dundee, Scotland). TRFLP profiles were analysed using GeneMapper 5.0 (Applied Biosystems Inc.). Peaks between 50 and 8000 fluorescent units in height, representing TFs longer than 60 bp were analysed using a bin width of 2 bp and the local southern method of peak calling. TF frequency was measured using peak area. Raw peak area data were transformed into proportional abundances to account for variation in the total amount of DNA among samples (Culman *et al.*, 2008). Singletons across all samples and peaks representing less than 5% of the total abundance on average across all samples were excluded to eliminate background noise (Culman *et al.*, 2008). In all data analyses, AM fungal species richness was taken as the number of TFs detected, while the proportion of each TF was used as a proxy for the relative abundance of each species.

### **2.4.3 Data Analysis**

#### *2.4.3.1 Sequence Analysis*

The sequences returned from GATC Biotech were used for phylogenetic analysis. ClustalW (Thompson *et al.*, 1994) was used for multiple sequence alignment of abundant sequences. . The genetic relationships were inferred using the Neighbor-Joining method (Saitou and Nei, 1987). *Geosiphon pyriformis* (Kütz.) F. Wettst. (Gehrig *et al.*, 1996) was used as an outgroup to the AM fungi and *Corallochytrium limacisporum* as an outgroup to all fungi (Vandenkoornhuyse *et al.*, 2002a). Phylogenetic support was calculated using nonparametric bootstrapping (Felsenstein, 1985) with 1000 pseudoreplicates. To determine whether further sampling effort would affect the results, species accumulation curves using individual-based rarefaction were computed using these sequences. Unless

otherwise indicated all analyses were conducted in the R statistical language using the *vegan* and *spatstat* libraries (R-Development-Core-Team, 2011).

#### *2.4.3.2 Spatial Analysis: Aboveground*

To estimate the degree of environmental heterogeneity perceivable by the AM fungal communities in this experiment, the JavaScript image analysis software ImageJ (Rasband, 1997) was used to analyse the aboveground spatial patterns of the host plant communities in the following manner: The two-dimensional structure of each individual plant in the aerial photographs of the plots was delineated, then, for each plant, the area occupied and the Cartesian co-ordinates of its centroid measured. Using these data, the degree of spatial autocorrelation of the plants, as a surrogate for environmental heterogeneity, was determined at various spatial scales. Ripley's K function was used to determine the degree of spatial autocorrelation of the plants at all spatial scales up to the size of each plot. The aboveground plant spatial data were analysed using Ripley's K function (Ripley, 1976), Moran's I spatial autocorrelation coefficient (Moran, 1950) and a modified Ripley's K function called the mark correlation function (Penttinen *et al.*, 1992).

#### *2.4.3.2 Spatial Analysis: Belowground*

Mantel *r* statistics based on Pearson's product-moment correlation coefficient were computed for each plot, using geographic distance matrices and community dissimilarity matrices and 9,999 permutations. Species diversity (No. of TFs) was calculated for each soil core, and then averaged by three spatial scales: soil core, plot and host plant species (all three replicate plots combined).

## 2.5 Results

### 2.5.1 Host plant spatial patterns

*Aboveground spatial patterns differ across host plant species*

#### 2.5.1.1 Moran's I coefficient

*Global spatial autocorrelation coefficient*

At the plot-level, the aboveground spatial patterns differed between host-plant species ( $F_{3, 8} = 5.2$ ,  $P = 0.028$ ), as determined by Moran's I coefficient of spatial autocorrelation. Differences in the degree and type (positive or negative) of spatial autocorrelation were detected, and three plots (Lv3, Cj2 and Cj3) displayed a significant deviation from spatial independence in the form of positive spatial autocorrelation (Fig. 2.2, table 2.3). A Tukey's multiple comparison of means procedure revealed that only *C. jacea* and *A. odoratum* significantly differ from each other ( $p = 0.023$ ); all other pairwise comparisons were not significant. Five of the six grass plots displayed negative autocorrelation, while all of the forb plots displayed positive autocorrelation, three of which were significant.

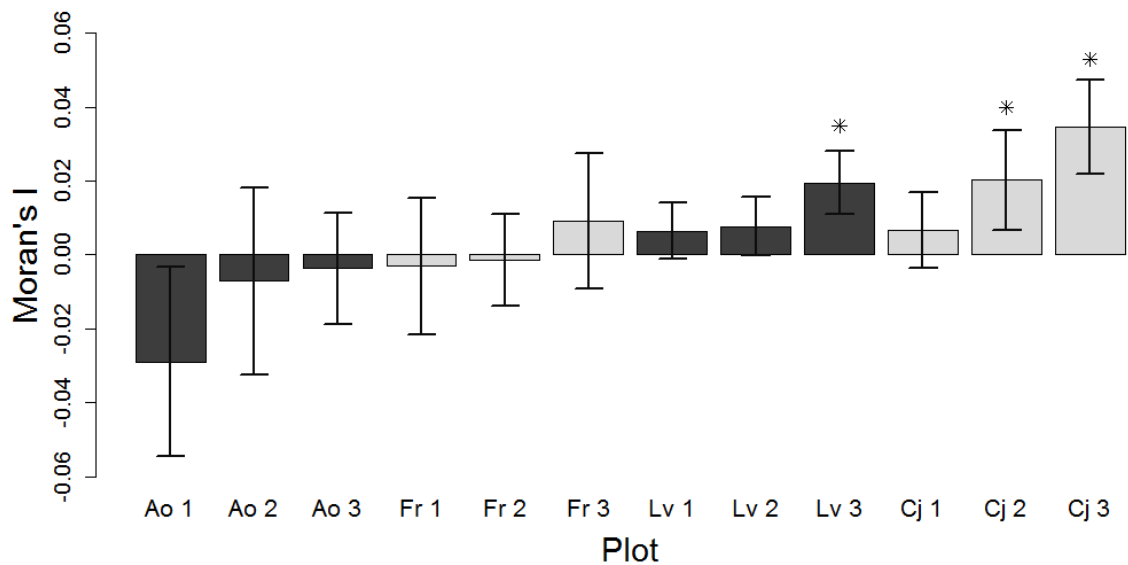


Figure 2.2. Moran's I coefficient of spatial autocorrelation for all monoculture plots. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*. Numbers after species abbreviations represent plot number. Bar height indicates degree of spatial autocorrelation either positive or negative. Positive values indicate positive spatial autocorrelation, or greater aggregation of plants than those under Complete Spatial Randomness (CSR), and negative values indicate negative spatial autocorrelation, or greater segregation, or uniformity, of plants than those under CSR. Error bars are standard deviation of Moran's I calculation. Asterisks above bars indicate significant deviation from the null hypothesis of no spatial autocorrelation.

### 2.5.1.2 Ripley's K function

#### *Small-scale spatial autocorrelation of host plant individuals*

Examination of host plant aboveground spatial patterns using Ripley's K function revealed the spatial scales within each plot at which each monoculture displayed positive or negative spatial autocorrelation (Fig. 2.3 (i), Table 2.1).

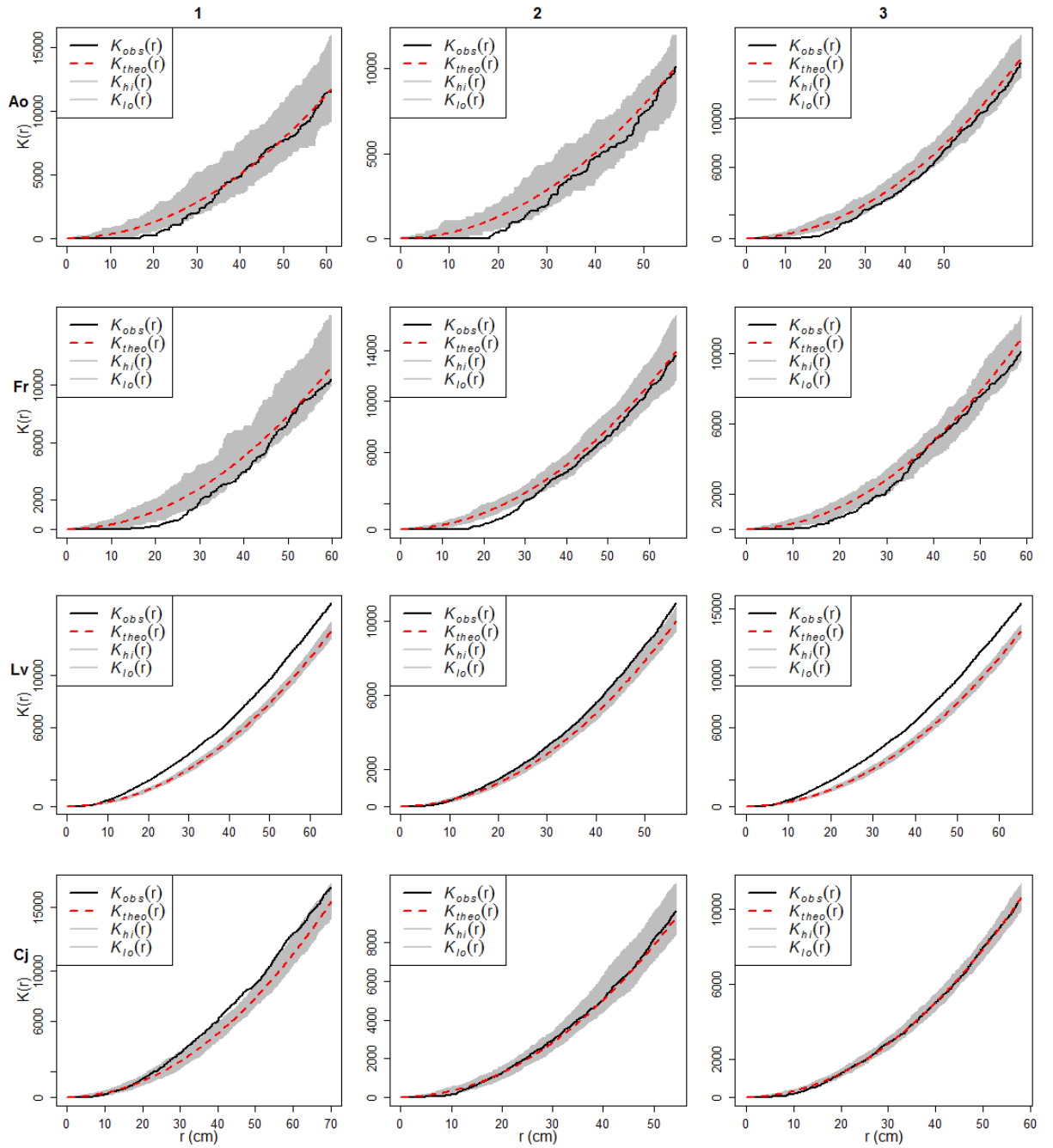


Figure 2.3. (i) Ripley's K function ( $K(r)$ ) for all monoculture plots. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*. Numbers at top represent plot number. Red dashed line represents a simulated population under Complete Spatial Randomness (CSR). Black line is the observed  $K(r)$ . Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.



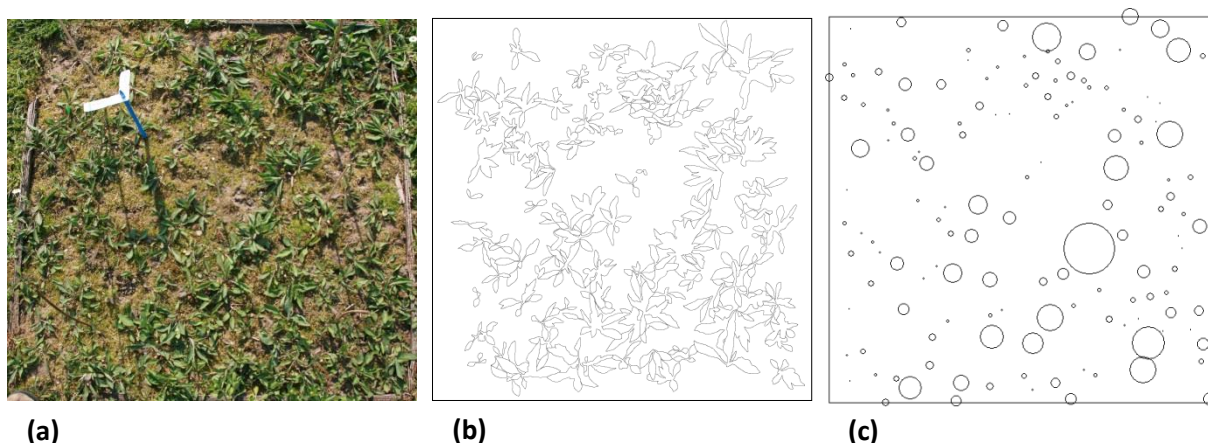


Figure 2.3. (ii) Aerial photograph (a), delineated two-dimensional structure of individual plants (b) and schematic diagram of individual plants (c), in which each circle represents one individual plant, the diameter of each circle is directly proportional to the area occupied by each plant and the centroid of each circle is in the same position as the centroid of the delineated two-dimensional structure of each plant, from *Centaurea jacea* plot Cj1.

Table 2.1. Summary of spatial autocorrelation trends as estimated by Ripley's K function for each plot

Plant species	Plot	Ripley's K
<b>A. odoratum</b>	<b>Ao1</b>	-
	<b>Ao2</b>	-
	<b>Ao3</b>	-
<b>F. rubra</b>	<b>Fr1</b>	-
	<b>Fr2</b>	-
	<b>Fr3</b>	-
<b>L. vulgare</b>	<b>Lv1</b>	+
	<b>Lv2</b>	+
	<b>Lv3</b>	+
<b>C. jacea</b>	<b>Cj1</b>	+
	<b>Cj2</b>	-
	<b>Cj3</b>	CSR

Both grass species display significant negative spatial autocorrelation at the smaller spatial scale of the plot (0 – 30 cm), but do not differ from complete spatial randomness (CSR) at any other scale within the plot. Two of the three *L. vulgare* plots (Lv1 and Lv3) display a significant and great degree of positive spatial autocorrelation at almost all scales, and the third (Lv2) is significantly positively spatially autocorrelated at scales above 30 cm, but to a much lesser degree than the other two plots. Only one of the *C. jacea* plots (Cj1) appears to display significant positive spatial autocorrelation at any scale. In contrast, the other two *C. jacea* plots (Cj2 and Cj3) display patterns of spatial autocorrelation similar to the grasses, showing significant negative spatial autocorrelation at small spatial scales (< 20 cm).

### 2.5.1.3 Modified Ripley's K: Mark correlation function

#### *Rate of decay in autocorrelation of host plant individuals at small spatial scales*

While Ripley's K function computes the observed spatial autocorrelation of the individual host plants compared to that expected under CSR, it does not include the size of each individual in its calculation; only their locations. A modified Ripley's K function, also known as the Mark correlation function, was used to estimate the rate of decay of similarity between individual host plants, in terms of plant size. This yielded a further, quantitative, measure of spatial autocorrelation at small spatial scales (smaller than an individual plot), from which spatial heterogeneity, as perceived by AMF, could be inferred. The Mark correlation function ( $K_{mm}(r)$ ) describes the degree to which values of the soil environmental variables are spatially autocorrelated at all distances incorporated within the study area. Values of the observed  $K_{mm}(r)$  greater than one indicate positive autocorrelation, or a mutual attraction, of the "mark" (in this case the soil environmental variable) under study at a distance 'r'. In this situation, values are more similar than would be expected in a spatially uncorrelated variable.  $K_{mm}(r)$  values less than one indicate negative spatial autocorrelation, or mutual inhibition, between marks, where values are more different (at a distance 'r') than would be expected in a spatially uncorrelated variable. Negative values indicate greater heterogeneity, therefore, because values at a certain distance are more likely to be significantly different from one another (Penttinen *et al.*, 1992). The rate of decay in autocorrelation across the plot differs between the grasses and the forbs. In the *A. odoratum* and *F. rubra* plots the distance at which the autocorrelation is no different from 1, indicating no significant autocorrelation, falls between 20 and 30 cm (Fig. 2.4). In the *L. vulgare* and *C. jacea*

plots, this distance is between 5 and 15 cm. This lower rate of decay of autocorrelation indicates a greater degree of spatial heterogeneity in the grass plots.

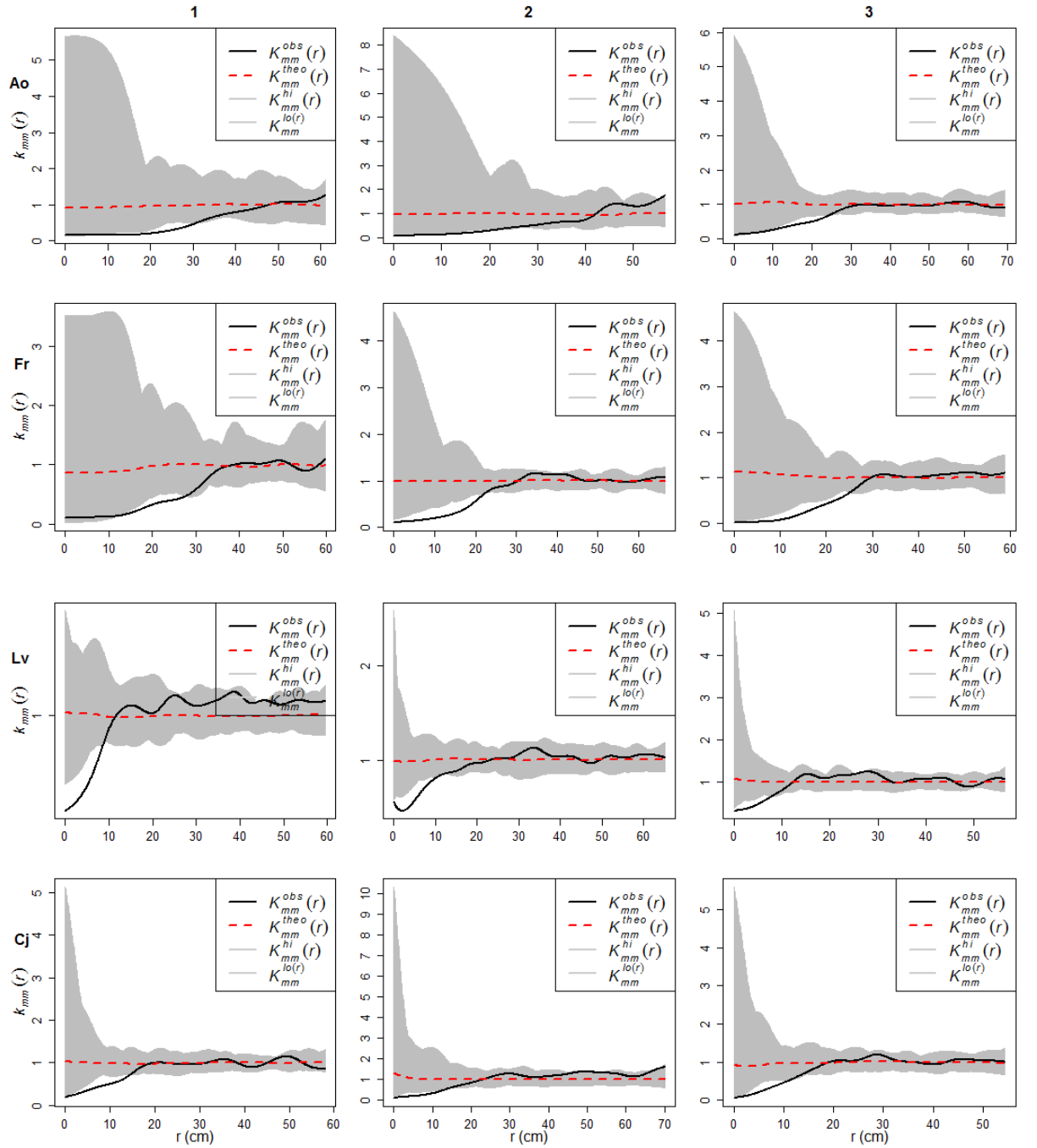


Figure 2.4. Correlograms of the mark correlation function for the size of individual plants for all monoculture plots. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*. Numbers at top represent plot number. Red dashed line represents a theoretical simulated population under Complete Spatial Randomness (CSR). Black line is the observed mark correlation function ( $K_{mm}(r)$ ). Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.

## 2.5.2 AM fungal community diversity and composition

### 2.5.2.1 Sequence analysis

Two-hundred and sixteen clones containing the SSU rDNA gene were screened using SP6-T7 amplification and purified and sequenced with the T7 universal primer. Using the neighbour-joining method, 15 AM fungal taxa were recorded from the 12 monoculture plots in this experiment (Fig. 2.5). Distinct AM fungal taxa were defined as sequenced types which were  $\geq 3\%$  different from all other AM fungal sequences recorded from natural field systems. No non-AM fungal sequences were obtained. The 15 AM fungal taxa recorded in this study are given in Fig. 2.5 and were from *Glomus* group A (Glomerales), *Glomus* group B (Glomerales) and Gigasporaceae (Diversisporales) following (Schüßler *et al.*, 2001). Species accumulation curves were computed using rarefaction. Rarefied species accumulation curves had reached an asymptote in three of the four host plant species in the experiment (Fig. 2.6)

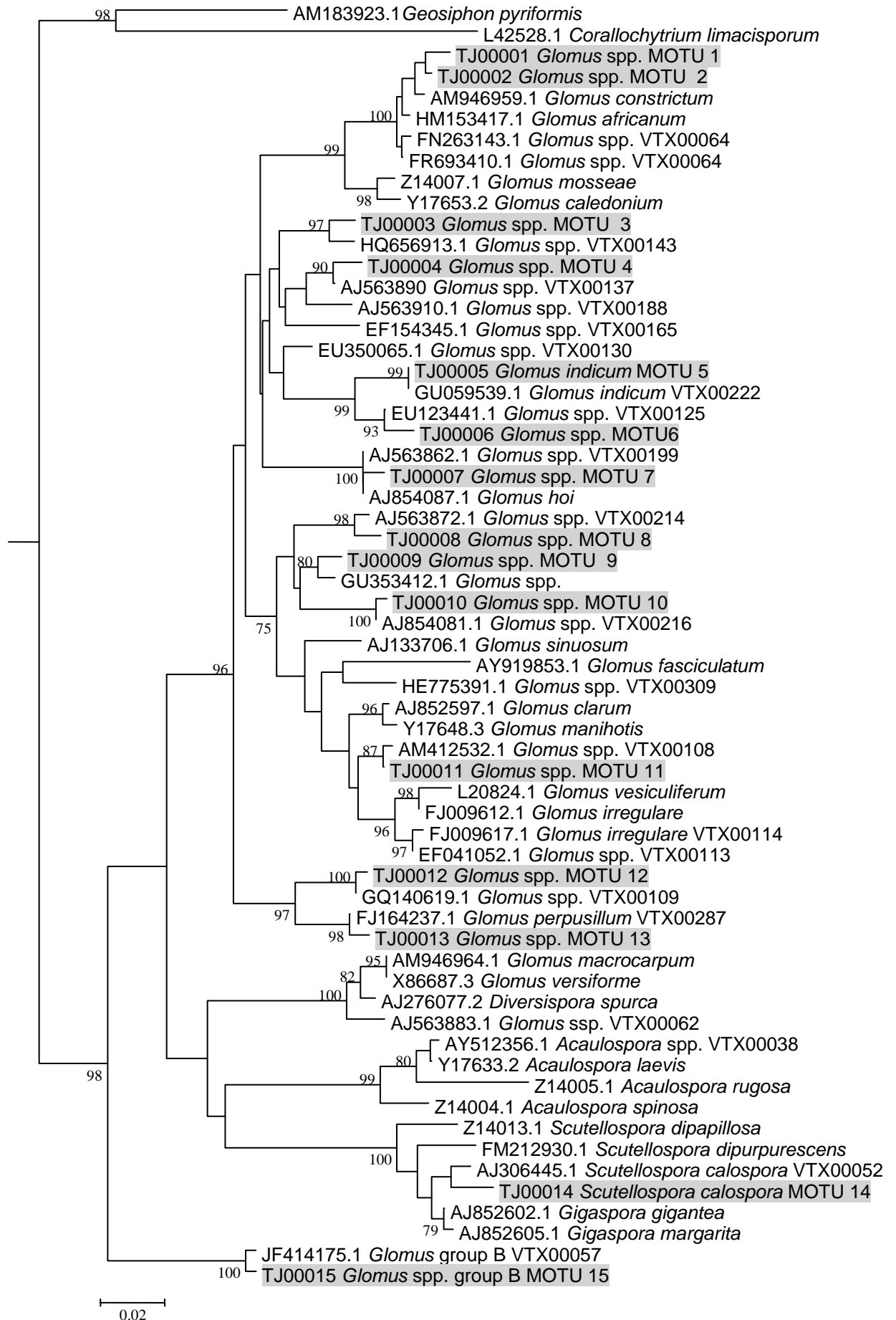


Figure 2.5. Neighbour-joining phylogenetic tree showing the arbuscular mycorrhizal (AM) fungal taxa from samples taken from the 12 monoculture plots (3 of each host-plant species) in the Wageningen Biodiversity experiment. Bootstrap values >75 % (1000 replicates) are shown above the branches and before the node to which they correspond. The endocytobiotic fungus *Geosiphon pyriformis* (Schüßler, 2002) was used as an outgroup to AM fungi and *Corallochytrium limacisporum*, a putative choanoflagellate, (Cavalier-Smith and Allsopp, 1996) was used as an outgroup to the fungi.

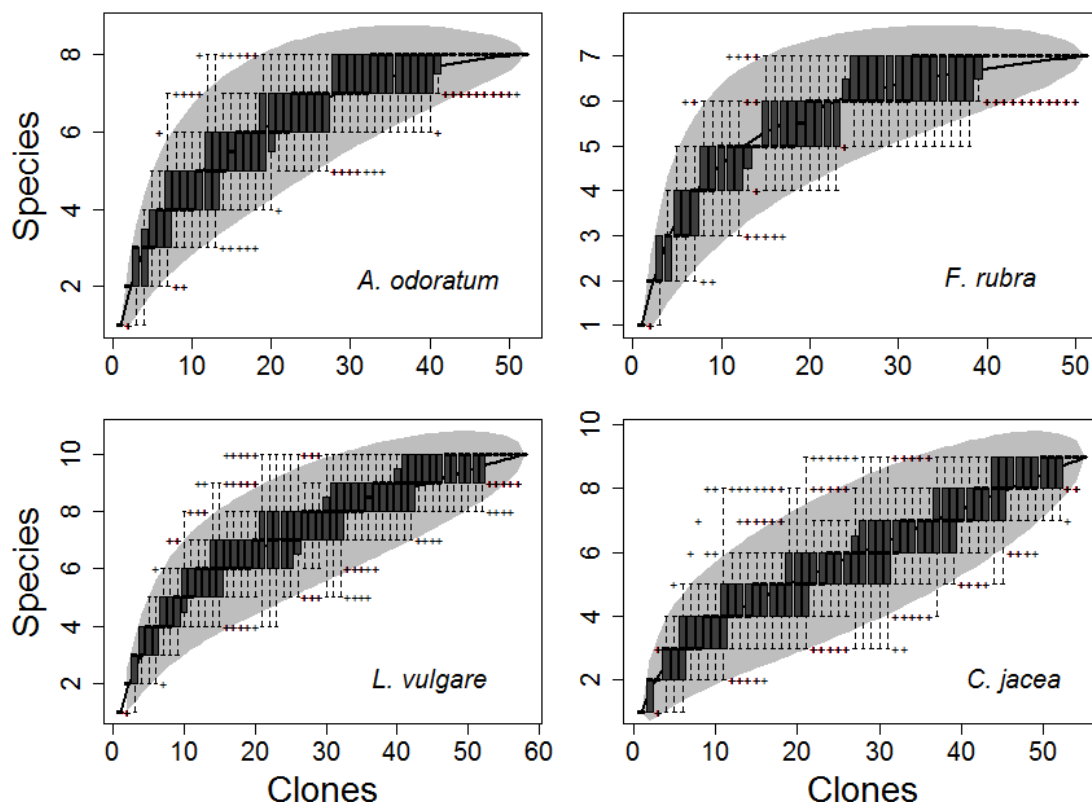


Figure 2.6. AM fungal species accumulation curves (light grey envelopes) for the four host plant species computed using individual-based rarefaction on clone library data. Boxplots display the mean and standard deviation of species richness per subset of clones.

### 2.5.2.2 TRFLP analysis

Of the 180 soil cores sampled from across the 12 plots, 121 yielded sufficient DNA for digestion and subsequent TRFLP profiling. TRFLP analysis detected 192 TFs ranging from 60 to 550 bp. Visual inspection of the rarefied TF-species accumulation curves showed TF-species accumulation had begun to asymptote when data were analysed per plot (Fig. 2.7) and per host plant species (Fig. 2.8). Thus, further sampling would be unlikely to qualitatively affect the results.

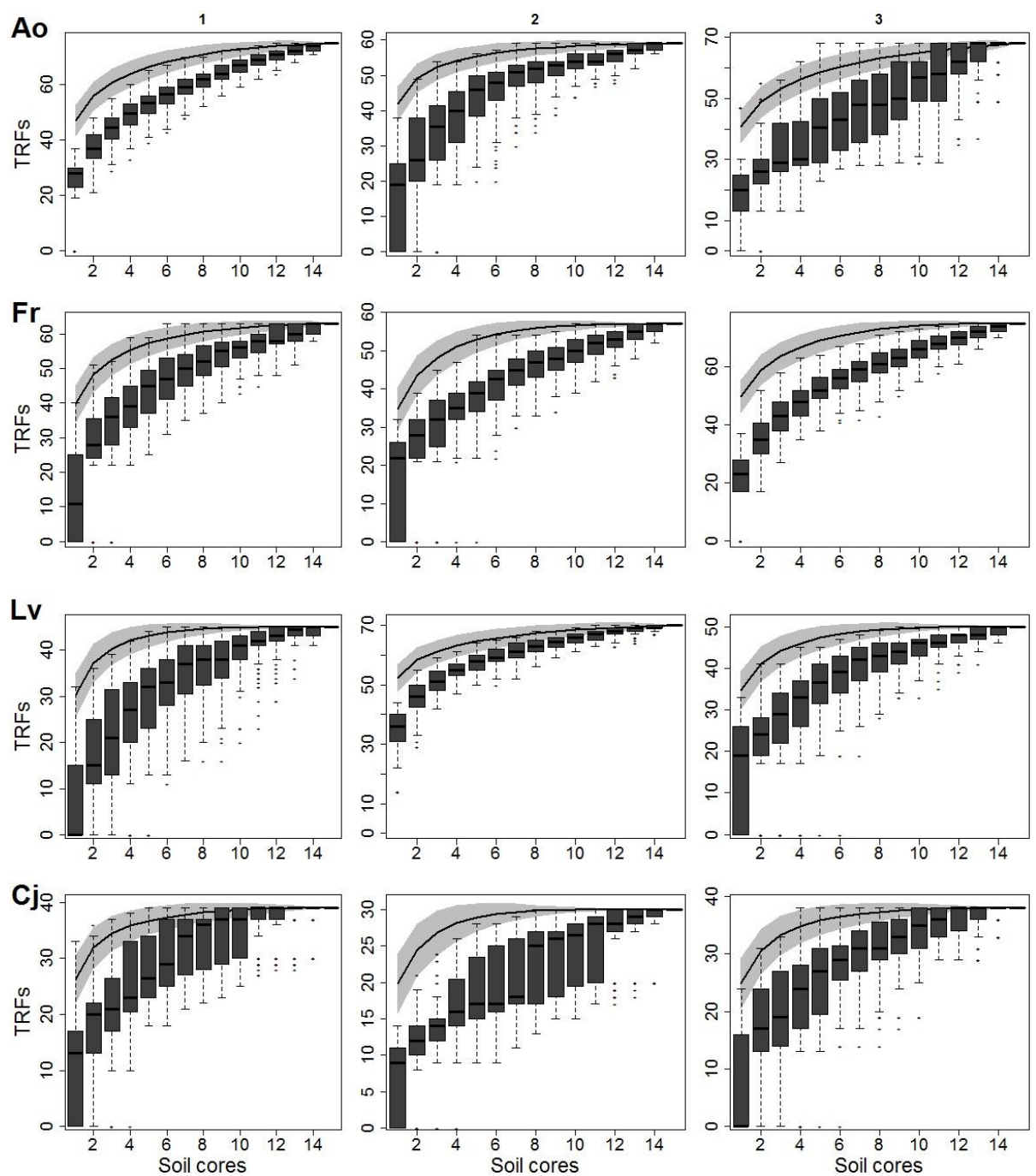


Figure 2.7. Terminal Fragment (TF)-Species accumulation (AM fungi) in the twelve plots computed using individual-based rarefaction on TRFLP data. Boxplots display the mean and standard deviation of TF-species richness per subset of soil cores.

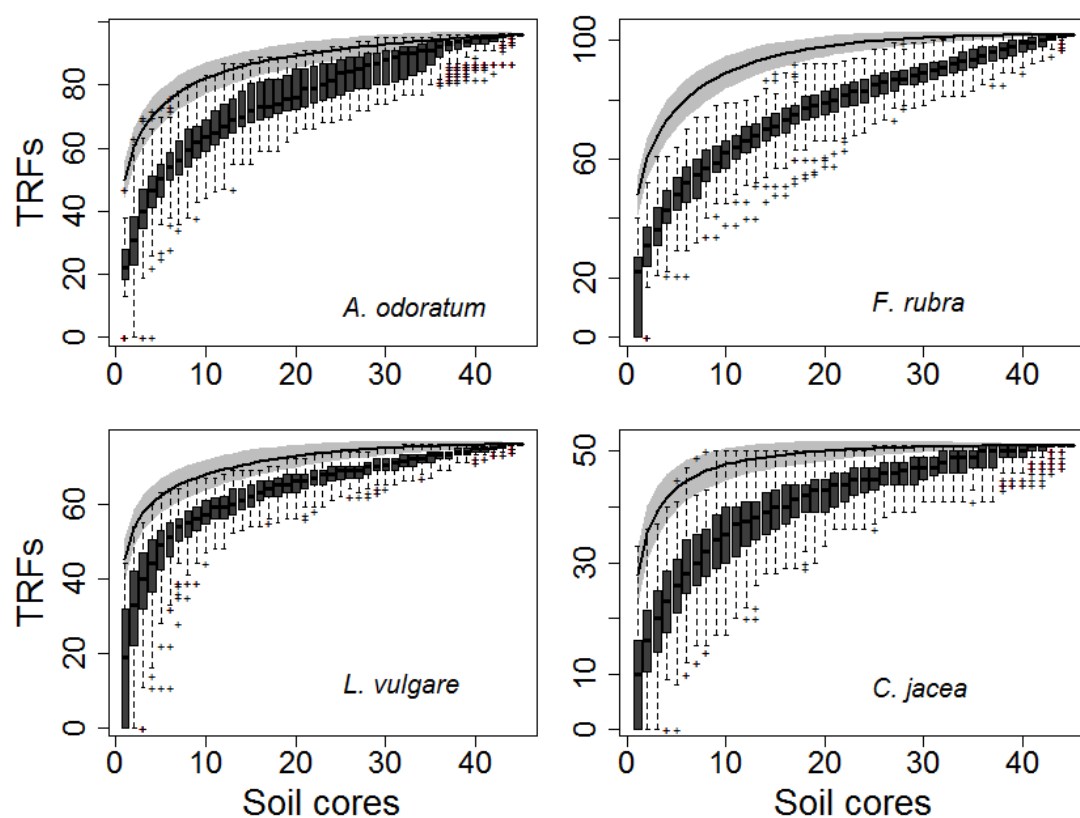


Figure 2.8. Terminal Fragment (TF)-Species accumulation (AM fungi) in the four host plant species computed using individual-based rarefaction on TRFLP data. Boxplots display the mean and standard deviation of TF-species richness per subset of soil cores.



## 2.5.3 Spatial scales

### 2.5.3.1 Core-level

When the mean AMF species richness was calculated at the core-level, the three *C. jacea* plots were among the four least species-rich plots in the entire experiment, along with one of the *L. vulgare* plots (Fig. 2.9). This led to the qualitative result of *C. jacea* hosting the least species-rich AMF communities at the core-level when pooled by host plant species (Fig. 2.10). The four plant species hosted AMF communities of significantly different species richness ( $F_{3, 176} = 6.28$ ,  $P < 0.001$ ). A Tukey's multiple comparison of means procedure revealed that this difference was driven by the significantly less species-rich AMF communities hosted by *C. jacea* compared with those of *L. vulgare*, *A. odoratum* and *F. rubra* ( $P = 0.004$ ,  $P = 0.002$  and  $P = 0.003$  respectively), none of which differed from each other.

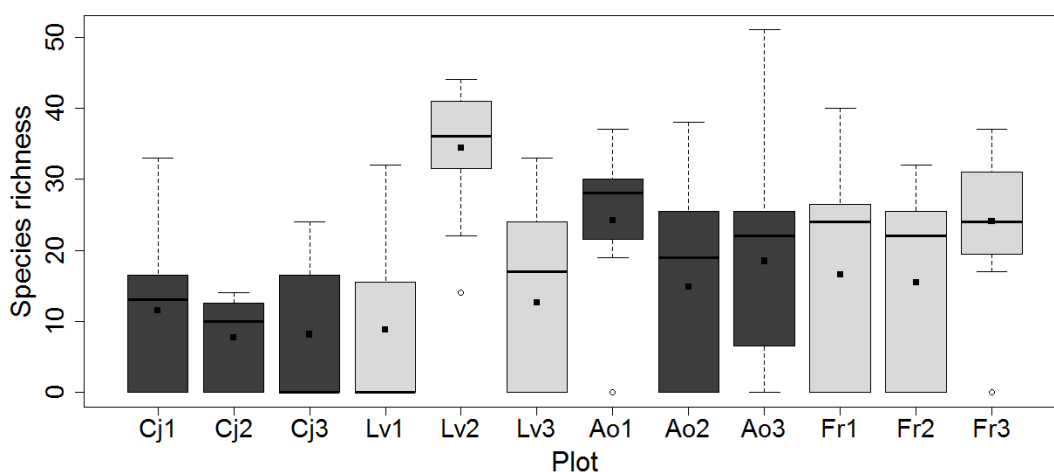


Figure 2.9. Terminal Fragment (TF)-Species richness (No. of TRFs detected) of cores within each plot. Black lines are median values. Black squares are means.

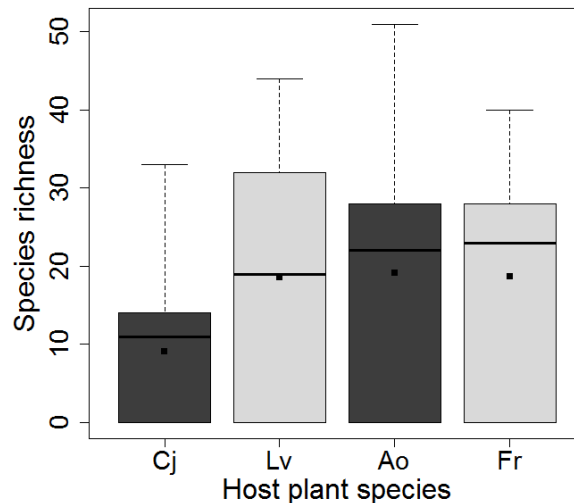


Figure 2.10. Terminal Fragment (TF)-Species richness (No. of TRFs detected) of cores from each host plant species. Black lines are median values. Black squares are means.

In a similar manner, a significant difference in AMF species diversity (inverse Simpson's diversity index) between host plant species at the core-level ( $F_{3, 176} = 5.93$ ,  $P < 0.001$ ) was detected (Fig. 2.11). A Tukey's multiple comparison of means procedure revealed that it was the species diversity of the communities associated

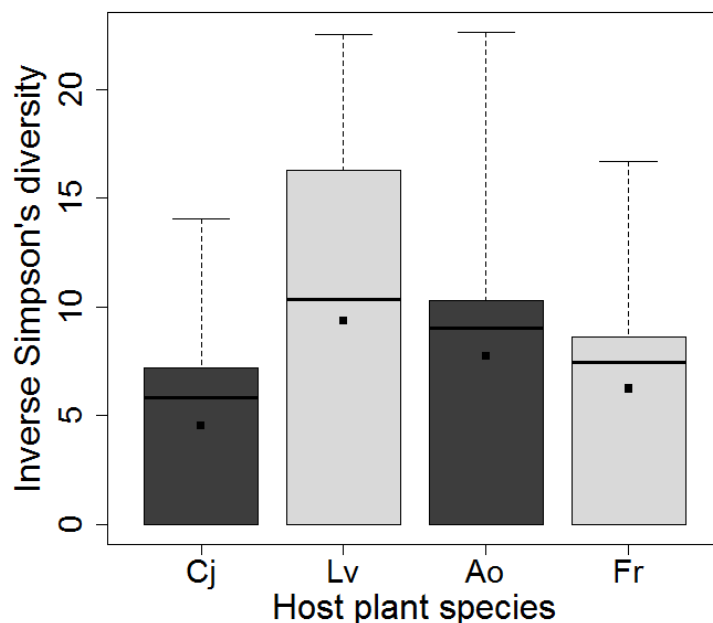
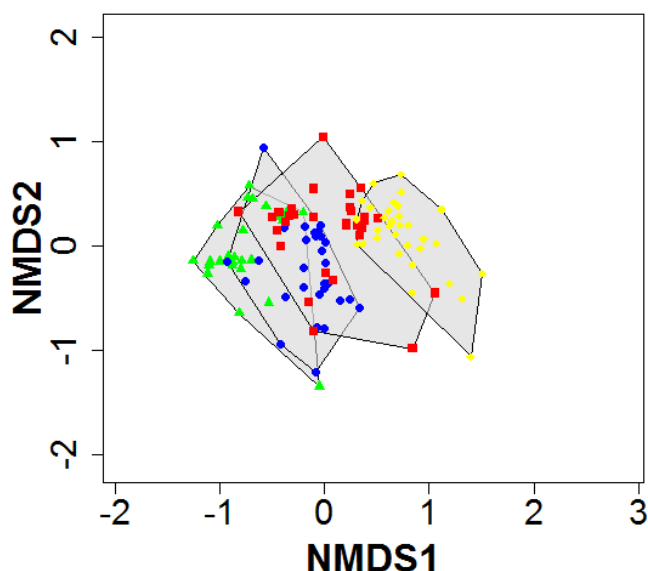


Figure 2.11. Inverse Simpson's diversity index on Terminal Fragment (TF)-Species of cores taken from each of the four host plant species. Black lines are median values. Black squares are means.

with *C. jacea* that differed from those associated with *A. odoratum* and *L. vulgare* ( $p = 0.04$  &  $p < 0.001$  respectively). Unlike the species-richness result, however, the *C. jacea* communities did not differ in diversity from those associated with *F. rubra* ( $p = 0.49$ ).

The compositional difference between AMF communities hosted by the four plant species at the core-level can be seen in Fig. 2.12. The *F. rubra* communities only overlap compositionally with *A. odoratum*, whereas *A. odoratum*, *C. jacea* and *L.*



*vulgare* all overlap. The compositional difference between the host plant species does not appear to be very great at the smallest spatial scale of the study. The separation between plant species, according to an ANOSIM analysis using the Bray-Curtis dissimilarity index was significant at the core level ( $R = 0.54$ ,  $p < 0.01$ ).

Figure 2.12. Nonmetric Multidimensional Scaling (NMDS) plot of all 122 cores from which TRFLP profiles were obtained. Coloured symbols indicate soil cores taken from plots of different host plant species. Red squares = *Anthoxanthum odoratum*, yellow diamonds = *Festuca rubra*, blue circles = *Leucanthemum vulgare* and green triangles = *Centaurea jacea*.

#### 2.5.3.2 Plot level

When the mean AMF species richness was calculated at the plot-level, the intermediate spatial scale of the study, a significant difference in AMF species richness between host plant species was detected ( $F_{3,8} = 7.3$ ,  $P = 0.01$ ) (Fig. 2.13). A Tukey's multiple comparison of means procedure revealed that at this larger spatial scale, the species richness of the communities associated with *C. jacea* differed from those associated with *A. odoratum* and *F. rubra* ( $P = 0.01$ ,  $P = 0.02$ ), but not from those associated with *L. vulgare* ( $P = 0.13$ ).

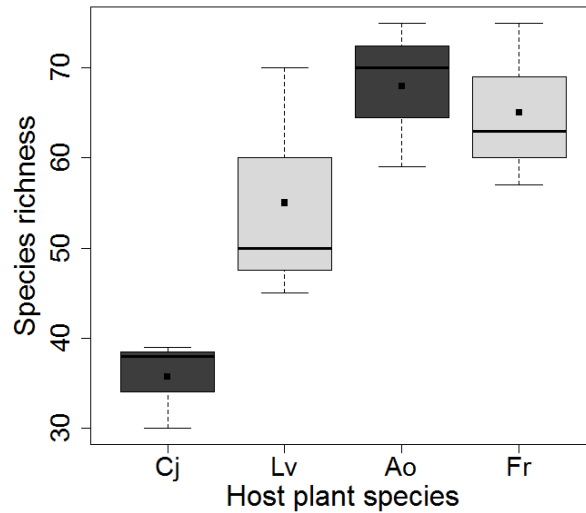
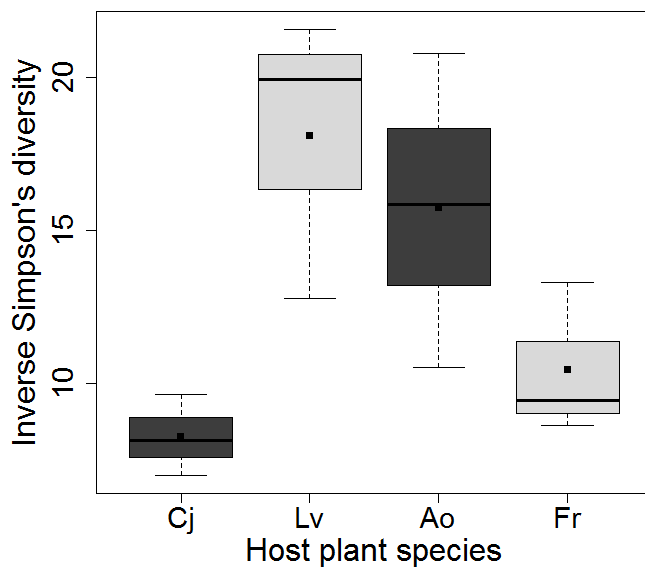


Figure 2.13. Terminal Fragment (TF)-Species richness (No. of TRFs detected) of plots from each host plant species. Black lines are median values. Black squares are means.

A significant difference in AMF species diversity between host plant species was detected ( $F_{3, 8} = 4.42$ ,  $P = 0.04$ )



(Fig. 2.14). A Tukey's multiple comparison of means procedure revealed that the only significant difference in diversity of AMF communities was between those detected in *L. vulgare* and those in *C. jacea* ( $P = 0.04$ ).

Figure 2.14. Inverse Simpson's diversity index on Terminal Fragment (TF)-Species of plots of each of the four host plant species. Black lines are median values. Black squares are means.

At the plot level, the *L. vulgare* and the *A. odoratum* plots seem to be compositionally most similar, while *C. jacea* and *F. rubra* plots appear to differ significantly from all other plots (Fig. 2.15). The separation between plant species, according to an

ANOSIM analysis using the Bray-Curtis dissimilarity index was significant at the plot level ( $R = 0.61$ ,  $p < 0.01$ ).

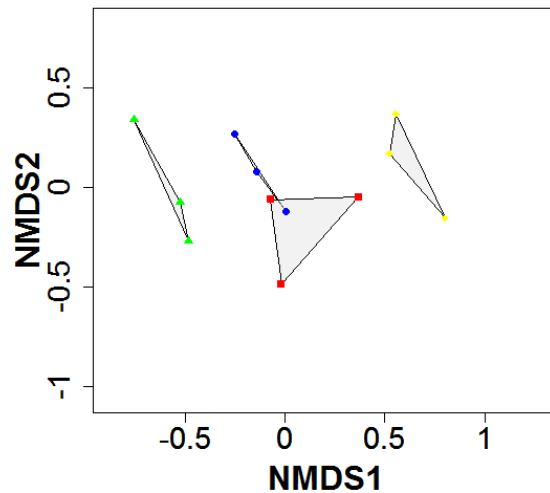


Figure 2.15. NMDS plot for all twelve plots in the study. Coloured symbols indicate soil cores taken from plots of different host plant species. Red squares = *Anthoxanthum odoratum*, yellow diamonds = *Festuca rubra*, blue circles = *Leucanthemum vulgare* and green triangles = *Centaurea jacea*.

### 2.5.3.3 Experiment level

At the largest spatial scale of the study, that of the three experimental plots for each host plant species combined, the *F. rubra* communities tested were the most species-rich, followed by those of *A. odoratum*, *L. vulgare*, then *C. jacea*, which hosted the least species-rich communities (Fig. 2.16).

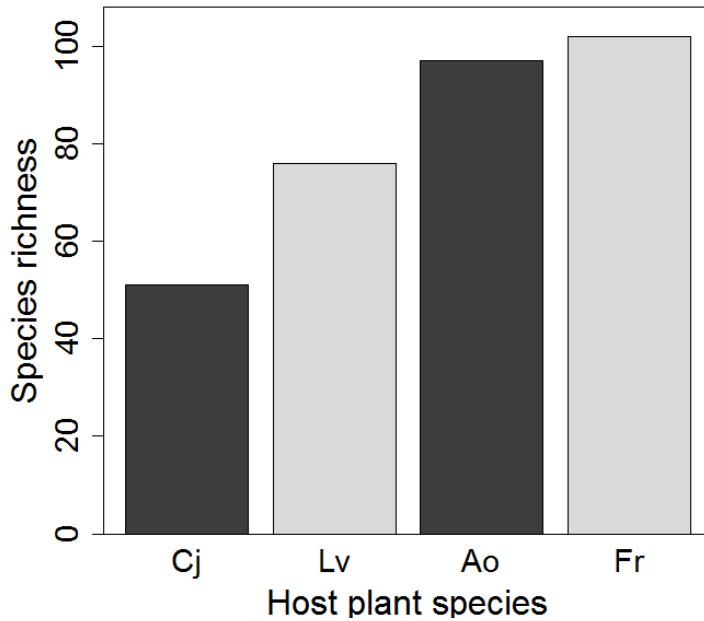


Figure 2.16. Total AM fungal species richness for all three replicate plots per host-plant species. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*.

*Anthoxanthum odoratum* hosted the most diverse AMF communities at the largest spatial scale, with a slightly greater Simpson's diversity index than *L. vulgare* (Fig. 2.17). The diversity of the *F. rubra* communities was greater, by a small degree, than those of *C. jacea*, which hosted the least diverse AMF communities. The similarity in AM fungal alpha diversity (Inverse Simpson's diversity index) for each pairwise combination of host plant species was computed and compared to 10,000 randomised community simulations to test for significance. Using the Bonferroni correction on the P values to reduce the probability of a type I error ( $P = 0.0083$  significance level), the only difference in Alpha diversity that was not significant at the experiment level was that between *A. odoratum* and *L. vulgare* ( $P = 0.012$ ). All other comparisons were significantly different. Similarly, the Morisita-Horn index was computed for the AM fungal communities associated with each pairwise comparison of host plant species, as a measure of Beta diversity. These were compared with the Morisita-Horn indices of 10,000 randomised community simulations to test for

significance. Using the Bonferroni correction on the  $P$  values, only the difference in Beta diversity between the AM fungal communities associated with *C. jacea* and *F. rubra*, and that between communities associated with *L. vulgare* and *F. rubra* were significant ( $P = 0.004$  and  $P = 0.0011$  respectively).

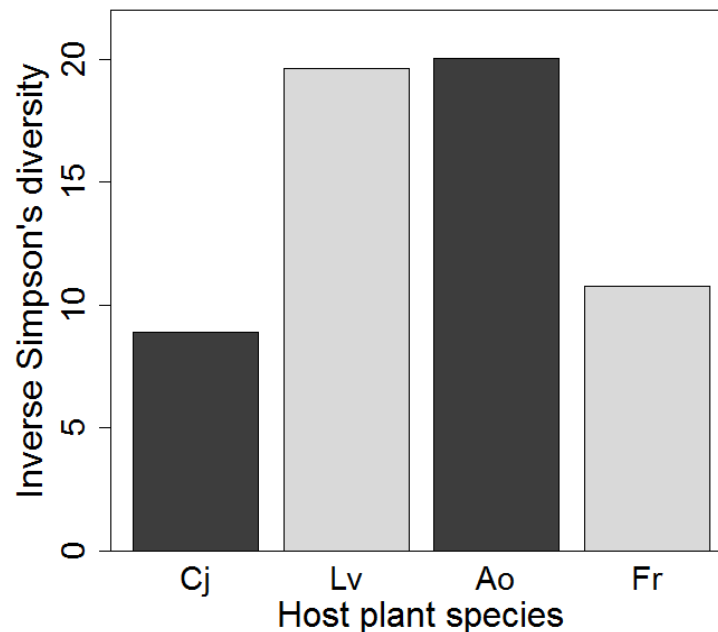


Figure 2.17. Total AM fungal species diversity (Inverse Simpson's diversity index) for all three replicate plots per host-plant species. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*.

At the largest spatial scale of the study, the compositional difference in AMF communities between the host plants is similar to that detected at the intermediate (plot-level) spatial scale. *L. vulgare* and *A. odoratum* are most similar to each other, while *C. jacea* and *F. rubra* differ from all the other plant species (Fig. 2.18).

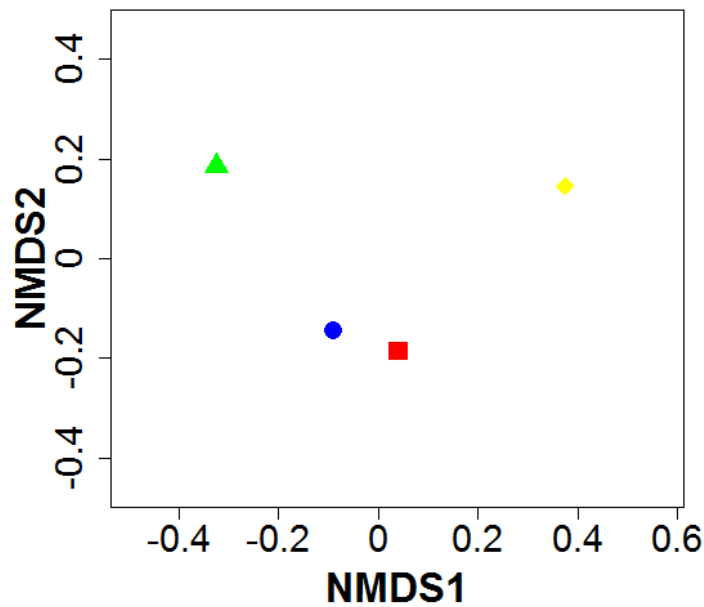


Figure 2.18. NMDS plot for all four host plant species in the study. Coloured symbols indicate the four different host plant species. Red square = *Anthoxanthum odoratum*, yellow diamond = *Festuca rubra*, blue circle = *Leucanthemum vulgare* and green triangle = *Centaurea jacea*.

#### 2.5.4 Scale-dependent patterns

Between core and plot level, the AMF species accumulation of *C. jacea* is greater than *L. vulgare*, evident from the fact that at the core level, the species richness of *L. vulgare* is significantly higher, but at the core level, they do not significantly differ. Similarly, between the core and the plot level, the AMF species accumulation in *A. odoratum* is greater than that in the *F. rubra* plots. However, between the plot and the experiment level, *F. rubra* displays greater species accumulation, as at the largest spatial scale, *F. rubra* hosts the most species-rich AMF communities.

The qualitative patterns in perceived AMF species diversity (Inverse Simpson's diversity index) remain the same over the two smallest spatial scales, but at the largest spatial scale, *A. odoratum* hosted the most diverse AMF communities.



Compositionally, the AMF communities associated with all four host plant species are more similar at the smallest spatial scale of the study, and become more different as the spatial scale increases (Figs. 2.12, 2.15 & 2.18). There seem to be no groupings based on Grasses vs forbs, with *A. odoratum* and *L. vulgare* the closest to each other compositionally, according to the NMDS plots.

#### 2.5.4.1 Distance decay and SARs

Five of the twelve plots in the study displayed significant distance decay according to the Pearson's product-moment correlation between the geographic distance matrix computed from the cores within each plot and the Euclidean community dissimilarity matrix computed from the structure and composition of the AMF communities within each core (Table 2.2, Fig. 2.19).

Table 2.2. Mantel test statistics for the 12 Host plant plots, with associated significance levels. Mantel test performed on Geographic distance matrix and AMF community Euclidean distance matrix. Bold text indicates a significance level of  $p < 0.05$ .

		Mantel statistic Significance	
<b>Ao</b>	<b>1</b>	0.11	0.19
	<b>2</b>	<b>0.30</b>	<b>0.02</b>
	<b>3</b>	<b>0.47</b>	<b>0.00</b>
<b>Fr</b>	<b>1</b>	<b>0.26</b>	<b>0.02</b>
	<b>2</b>	0.19	0.06
	<b>3</b>	0.02	0.45
<b>Lv</b>	<b>1</b>	-0.06	0.64
	<b>2</b>	<b>0.28</b>	<b>0.03</b>
	<b>3</b>	-0.04	0.58
<b>Cj</b>	<b>1</b>	-0.06	0.75
	<b>2</b>	0.17	0.06
	<b>3</b>	<b>0.35</b>	<b>0.01</b>

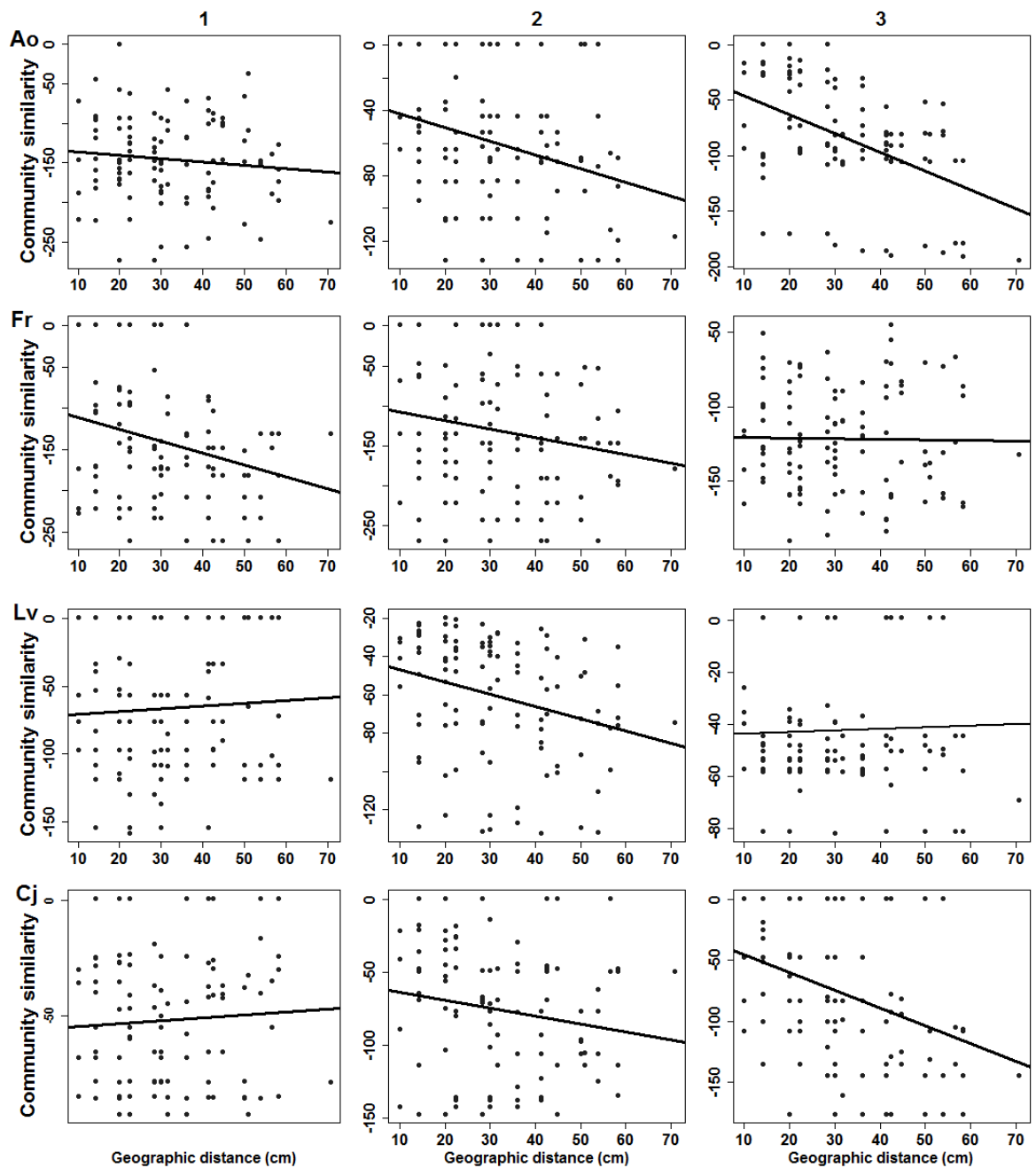


Figure 2.19. Distance decay in the AMF community similarity between the cores in each of the twelve plots in the study. Lines are linear regression between the geographic distance matrix computed for the position of the cores on each plot and the Euclidean distance matrix based on AMF community dissimilarity. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*. Numbers at top represent plot number.

The species-area relationships for each plot display clear spatial-scale dependences within the scale of each plot (Fig. 2.20). The linear regression curves on simulations of species-area relationships for different host plant species intersect, as do some of those for different plots of the same host plant species (Fig. 2.20).

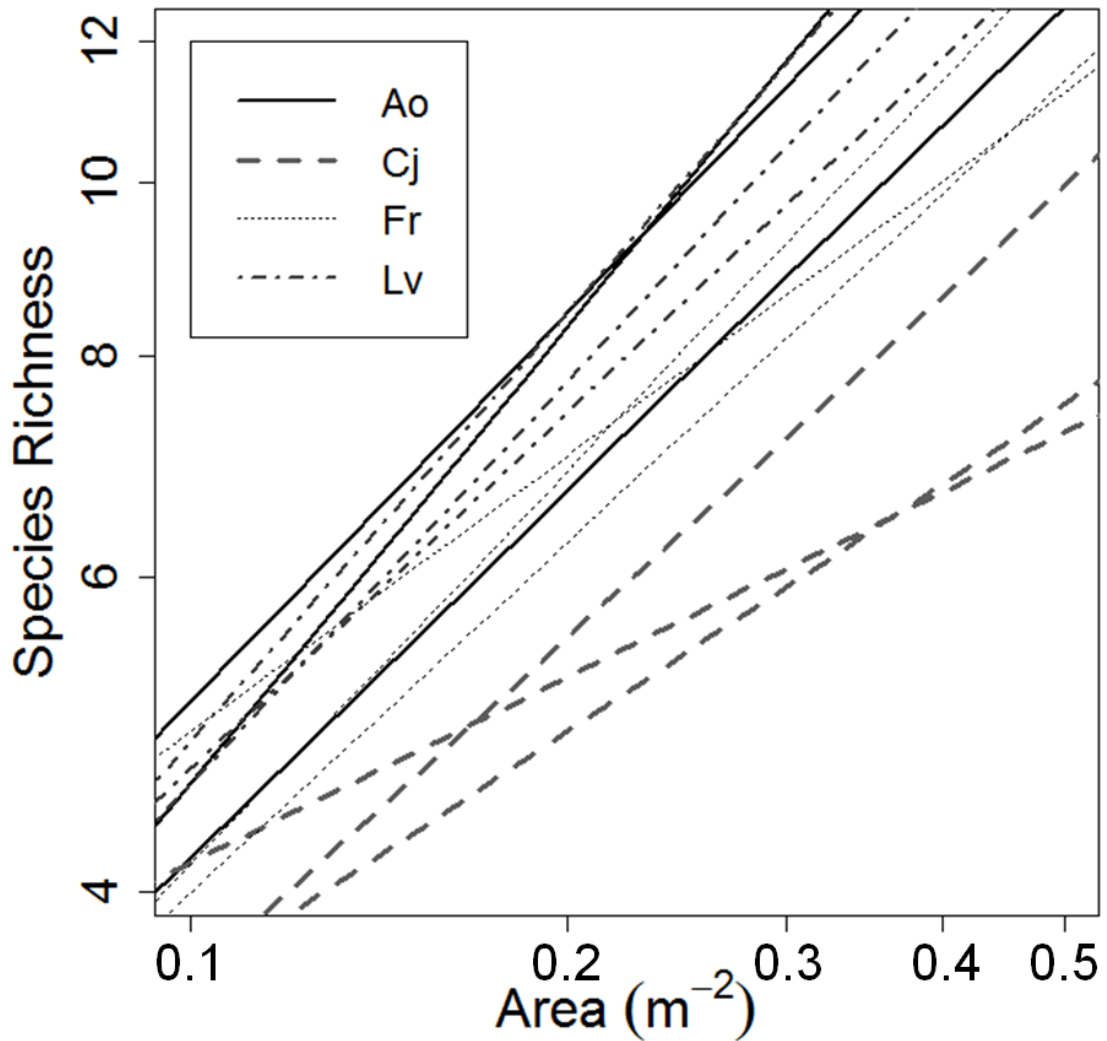


Fig. 2.20. Linear regression on simulations of species-area relationships for each plot. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*.

#### 2.5.4.2 Aboveground-belowground linkages

Those plots that displayed greater levels of spatial heterogeneity were not consistently the same plots in which significant distance decay was detected (Figs. 2.2 - 2.4 & 2.19, Table 2.3). Only *L. vulgare* and *C. jacea* displayed significant positive spatial autocorrelation of their aboveground parts according to both Moran's I and Ripley's K functions (Figs. 2.2 & 2.3), and these species also displayed a greater degree of heterogeneity in individual plant size within each plot (Fig. 2.4). Significant distance decay relationships in AM fungal community similarity were

detected in at least one plot per host plant species, however (Tables 2.2 & 2.3, Fig. 2.19).

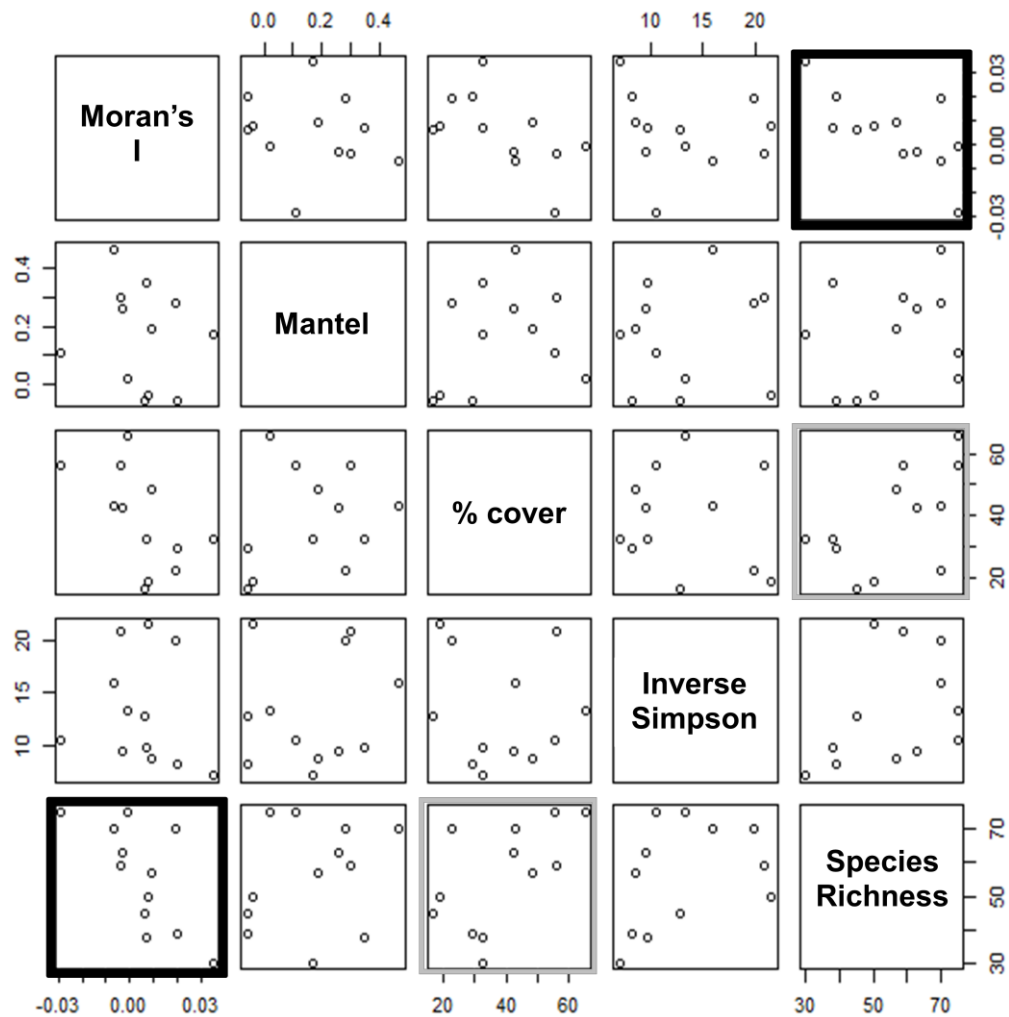


Figure 2.21. Scatterplot matrix for the 12 host-plant monoculture plots. Correlation between Moran's I coefficient of spatial autocorrelation, Mantel test statistic, percentage cover of host plants, inverse Simpson's diversity index and Terminal Fragment (TF) Species richness. Plots delineated by black square significant correlation ( $P = 0.01$ ). Plots delineated by grey square approaching significance ( $P = 0.051$ ). See text for results of regression analysis and model fitting.

Of the correlations tested, only that between Moran's I coefficient of autocorrelation and AMF species richness was the only significant relationship (Fig. 2.21). AMF community species richness for each plot significantly negatively correlated with

Moran's I coefficient of autocorrelation ( $F_{1,10} = 9.7$ ,  $P = 0.01$ ).  $R^2 = 0.49$ . A weak, non-significant, positive correlation exists between AMF species richness and percentage of each plot that is covered by the host plant ( $F_{1,10} = 4.94$ ,  $P = 0.051$ ).  $R^2 = 0.33$ . The AMF species richness and the percentage of each plot that is covered by the host plants' aboveground parts can be seen in Fig. 2.22.

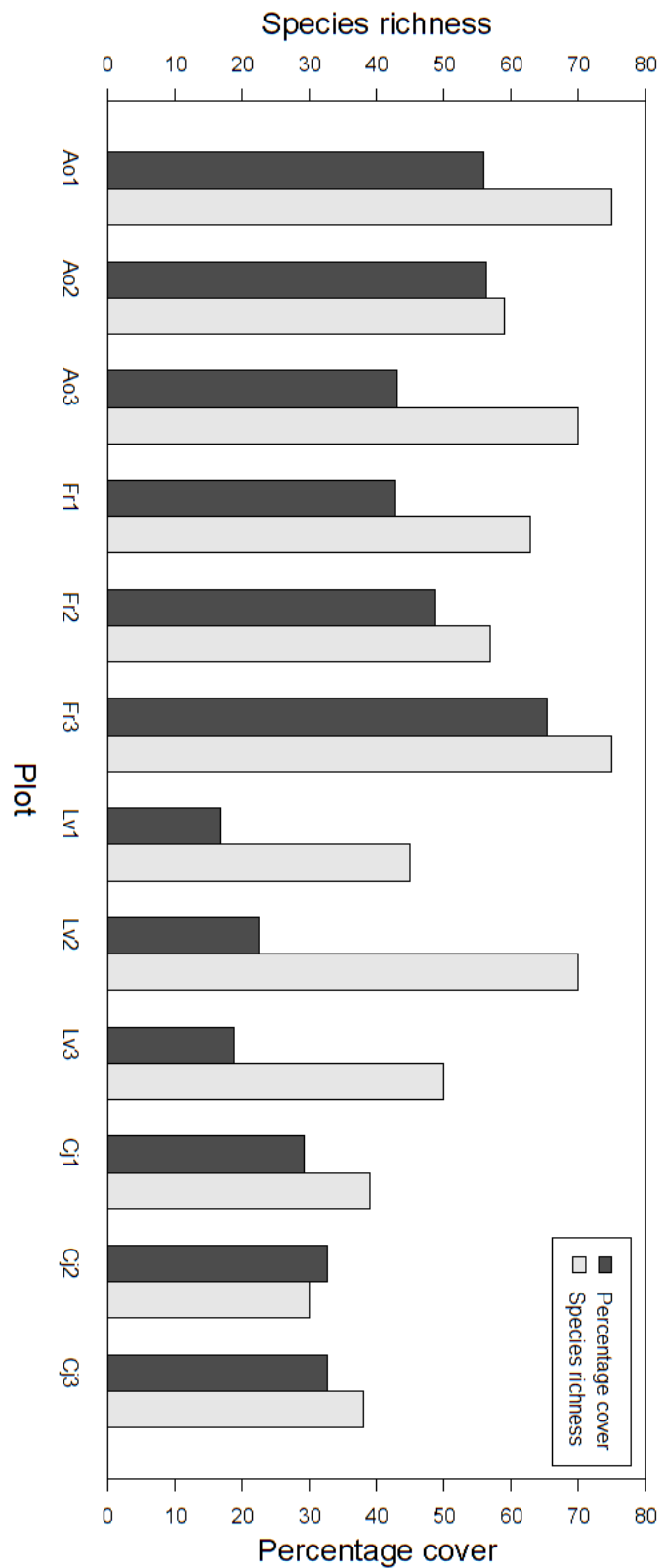


Figure 2.22. AM fungal Terminal Fragment (TF) species richness and percentage cover of host plant per plot. Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, Lv = *Leucanthemum vulgare* and Cj = *Centaurea jacea*. Numbers after species abbreviations represent plot number.

Table 2.3. Summary of spatial autocorrelation coefficient types and trends, Mantel test statistic, percentage of each plot covered by host plant, Inverse Simpson's diversity index and AMF species richness. Grey highlighted values indicate statistically significant values.

Plant species	Plot	Ripley's K	Moran's I	Mantel	% cover	Simpson	spp. Richness
<b><i>A. odoratum</i></b>	<b>Ao1</b>	-	-0.029	0.11	55.9	10.53	75
	<b>Ao2</b>	-	-0.007	<b>0.30</b>	56.4	20.78	59
	<b>Ao3</b>	-	-0.004	<b>0.47</b>	43.0	15.87	70
<b><i>F. rubra</i></b>	<b>Fr1</b>	-	-0.003	<b>0.26</b>	42.7	9.47	63
	<b>Fr2</b>	-	-0.001	0.19	48.6	8.64	57
	<b>Fr3</b>	-	0.009	0.02	65.5	13.30	75
<b><i>L. vulgare</i></b>	<b>Lv1</b>	+	0.006	-0.06	16.6	12.78	45
	<b>Lv2</b>	+	0.008	<b>0.28</b>	22.4	19.92	70
	<b>Lv3</b>	+	<b>0.019</b>	-0.04	18.8	21.57	50
<b><i>C. jacea</i></b>	<b>Cj1</b>	+	0.007	-0.06	29.2	8.16	39
	<b>Cj2</b>	-	<b>0.020</b>	0.17	32.6	7.02	30
	<b>Cj3</b>	CSR	<b>0.035</b>	<b>0.35</b>	32.7	9.66	38

## 2.6 Discussion

### 2.6.1 Host plants as a source of environmental heterogeneity

*Plant species vary in the degree of environmental heterogeneity perceived by AM fungi*

The spatial autocorrelation of the plant individuals on each plot was compared to the associated AM fungal communities. In estimating the spatial autocorrelation of host plant individuals by analysing the aboveground plant parts, the assumption is one of synchronicity and spatial correlation between shoots and roots. Root and leaf production, while not causally connected exclusively to one another, share strong and significant physiological links. A significant positive correlation between root and shoot phenology has been recorded to the greatest degree in grassland systems such as the one used in the current study (Steinaker and Wilson, 2008). Given the relative lack of spatiotemporal disparity between roots and shoots in this experimental design, and the dependence of AM fungi on the presence of plant roots

(Smith and Read, 2008), it follows that the aboveground spatial patterning of the host plants represents a source of environmental heterogeneity potentially perceptible by associated AM fungal communities.

Differences in spatial patterns of the host plants were more apparent between the ecological guilds of Grasses and Forbs than between species within ecological guilds. Such differences in spatial patterns between grasses and forbs were observed with each of the four metrics used in their analysis. Plots planted with the grass species were predominantly negatively spatially autocorrelated according to Ripley's K and Moran's I functions, and were less heterogeneous than the forb plots according to the mark correlation function. The grass plots also had significantly greater coverage of vegetation as a percentage of the entire plot than the forb plots. The forb plots were predominantly positively spatially autocorrelated according to Ripley's K function and entirely positively spatially autocorrelated according to Moran's I coefficient of autocorrelation (three plots significantly so) (Table 2.3, Figs 2.2 - 2.4 & 2.22). The grasses and the forbs in this study, therefore, differ in the degree of environmental heterogeneity, in terms of root abundance (hence habitat availability), as perceived by AM fungi. Within ecological guilds, however, there appeared to be little difference in aboveground spatial patterns between host plant species. This difference is most likely driven by differences in root morphofunctional traits between grasses and forbs.

### **2.6.2 Scale dependence of AM fungal community composition, diversity and structure**

*Different plant species host different AM fungal communities, observable only at certain spatial scales*



As predicted, the AM fungal communities associated with each of the four host plants are compositionally more similar at the smallest spatial scale of the study than they are at any of the larger scales (Figs. 2.12, 2.15 & 2.18). The separation between plant species, according to ANOSIM using the Bray-Curtis dissimilarity index was significant at both the core and the plot levels, but was greater in the latter ( $R = 0.61$ ,  $p < 0.01$ ) than the former ( $R = 0.54$ ,  $p < 0.01$ ). This indicates that observed similarity in AM fungal community composition between host plants is highly spatial-scale dependent. Therefore, the spatial grain and extent selected in the experimental design is likely to have an impact on whether or not AM fungal communities from different host plant species are reported as distinct from each other. Patterns in alpha diversity and species richness also differ between the spatial scales. At the two smaller scales, *A. odoratum* hosts the most species-rich AM fungal communities, followed by *F. rubra*, then *L. vulgare* and finally *C. jacea*. At the largest spatial scale, however, the order of highest to lowest AM fungal species richness is different, with *F. rubra* hosting the most species-rich AM fungal communities, followed by *A. odoratum*, then *L. vulgare* and *C. jacea* (Figs. 2.10, 2.13 & 2.16). This suggests a greater rate of AM fungal species accumulation for *F. rubra* between the plot-level and experiment-level of this study. Indeed, the SAR simulation for one of the *F. rubra* plots has a steeper slope than two of the three *A. odoratum* plots, potentially providing the greater rate of AM fungal species accumulation that results in the spatial scale-dependent patterns in species richness between *A. odoratum* and *F. rubra* (Fig. 2.20). In a similar scale-dependent manner, the most diverse AM fungal community at the core and plot levels of the study, as calculated using Inverse Simpson's diversity index, was that associated with *L. vulgare*, followed by the *A. odoratum*, *F. rubra* and *C. jacea* AM fungal communities

respectively. At the experiment level, the order of highest to lowest AM fungal diversity is different. At this largest spatial scale of the study, *A. odoratum* hosts the most diverse AM fungal community, followed by *L. vulgare*, *F. rubra*, and then *C. jacea*.

Crucially, the only significant differences in AM fungal alpha diversity detected at the two smaller spatial scales were those between *C. jacea* and *A. odoratum* and *L. vulgare* (core level) and between *C. jacea* and *L. vulgare* (plot level). At the largest spatial scale, a divergence in levels of diversity was observed; with the only pairwise comparison that was not significantly different was that between *A. odoratum* and *L. vulgare*.

These results indicate that the response of AM fungal diversity to host plant identity is potentially dependent on the spatial scale of sampling. However, given the intersecting lines of regression on the simulations of SARs for each plot (Fig. 2.20), there is no clear interspecific difference in the slopes of the SARs, except perhaps for those simulated for the *C. jacea* plots, which are generally less steep than those for the other host plant species. Apart from this exception, the slopes of the SARs seem to be idiosyncratic to each plot. This indicates spatial heterogeneity at the level of the whole experiment. Despite this lack of clear interspecific differences in SARs, it is evident that as the spatial scale increases, there is a divergence in the species richness of each AM fungal community, as they tend to be more similar in species richness at small spatial scales than at larger spatial scales. This pattern is similar to that recorded by Dumbrell *et al* (2008) in their study about scale dependent responses of diversity to disturbance, in which differences in diversity between disturbed and non-disturbed habitats were only observed at the largest spatial scale of the study.

### **2.6.3 Effect of environmental heterogeneity on spatial scaling of AM fungal community diversity and composition**

*The perceived environmental heterogeneity affects how AM fungal community diversity and composition scale through space*

Contrary to expectation, a significant negative correlation between Moran's I coefficient of spatial autocorrelation and AM fungal species richness was observed. The grasses displayed both greater levels of negative spatial autocorrelation and greater AM fungal species richness than the forbs. Also, only one of the twelve plots displayed both significant positive spatial autocorrelation and significant distance decay in AM fungal community similarity (Table 3). This could indicate one of three possible mechanisms: (1) that the autocorrelation of aboveground host plant spatial patterns does not represent a significant proportion of the heterogeneity to which the AM fungal communities are subject; (2) that negative spatial autocorrelation in host plants facilitates greater heterogeneity as perceived by AM fungal communities, or (3) that some parameter other than environmental heterogeneity drives greater AM fungal species richness in this grassland system.

The second strongest correlation of the parameters measured (Fig. 2.21, Grey Square) was that between AMF species richness and the percentage of each plot that is covered by the host plant. This was a positive correlation, approaching significance. Additionally, the two forb plots (Lv2 and Cj3) that displayed significant distance decay in AM fungal community similarity between cores, according to the Mantel test, were also those plots with the greatest percentage covered by the host plant, for *L. vulgare* and *C. jacea* respectively. Those forb plots with lower percentage cover displayed no significant distance decay. This suggests that the

percentage cover of the host plant covaries with parameters that affect how AM fungal community structure scales through space. Percentage cover of host plant can, in this case, be used as a surrogate for host plant-root biomass within each plot. Given the dependence of AM fungi on the presence of roots in order to survive and reproduce, the root biomass in a given area equates to the amount of available habitat. In those plots within this study with a greater host plant-root biomass, there is a greater probability that these roots will themselves inhabit a wider variety of environmental conditions throughout the soil. Hence those plots with a greater percentage cover of host plants are likely to exhibit a greater degree of environmental heterogeneity as perceived by the associated AM fungal communities. Additionally, given the positive correlation between root and shoot spatial occurrence in such systems as this, the host plant-roots of those plots whose plant populations are more uniformly distributed (i.e. negatively spatially autocorrelated) are more likely to cover a greater proportion of the belowground space available within each plot. Negative spatial autocorrelation in aboveground plant patterns, therefore, is also likely to result in greater environmental heterogeneity as perceived by AM fungi.

Environmental parameters whose variability and/or complexity (Li and Reynolds, 1995) could represent significant environmental heterogeneity perceptible by AM fungal communities include soil pH (Dumbrell *et al.*, 2011), soil organic matter (Joner and Jakobsen, 1995), availability of certain plant macronutrients in soil (Blanke *et al.*, 2005, Dumbrell *et al.*, 2010b) and soil moisture content (König *et al.*, 2010, Kivlin *et al.*, 2011). It has been reported that environmental heterogeneity influences AM fungal communities to a greater degree than host plant identity (Cheeke *et al.*, 2015). The current study provides evidence for the hypothesis that negative spatial

autocorrelation of aboveground plant parts can promote greater heterogeneity as perceived by AM fungal communities. This is due to the more evenly distributed plant-roots existing within a wider range of soil environmental parameters and thus increasing the heterogeneity of the environment which AM fungi can potentially inhabit.

#### **2.6.4 Scale dependence of host plant preference**

*Observed host-plant preference is spatial-scale dependent*

The intersecting lines of regression on the simulations of SARs for each plot (Fig. 2.20) and the greater degree of compositional overlap between AM fungal communities from the different host plant species at the smallest spatial scale indicate that the spatial scale of sampling can affect whether or not the AM fungal community from one host plant species is a subset of another. Given the divergence of AM fungal community composition, diversity and structure as the spatial scale of sampling increases, non-random AM fungal-host plant associations are more likely to be detected at the larger spatial scales of this study. If the differences in AM fungal community diversity, composition, species richness and species accumulation were smaller between the host plant species, then a detectable host plant preference in AM fungi would be less likely. It seems that the amount of root biomass within a certain area mediates spatial heterogeneity as perceived by AM fungi, by facilitating the existence of available AM fungal habitat within a wider range of soil environmental variables. The fact that *C. jacea* is the only non-rhizomatous host plant species in this study and hosted a less diverse, less species-rich AM fungal community than the other host plant species provides evidence in support of this hypothesis. Host plant preference in AM fungi, therefore, is more likely to be

dependent on spatial scale when comparing two or more host plants with rooting patterns and aboveground spatial patterns that result in a different proportion of the available belowground space to be occupied.

#### **2.6.5 Experimental limitations and further work**

At spatial scales larger than that encompassed by this study, the AM fungal diversity and species richness of the communities within each host plant monoculture would possibly continue to diverge, as differences in the absolute root biomass and thus the degree of environmental heterogeneity to which the AM fungal communities are subject will increase further. Indeed, that the differences in AM fungal community diversity, richness and composition were more pronounced at the larger spatial scales of this study suggests that this is the case. A recent study by Cheeke *et al.* (2015) found that at spatial scales greater than 1 metre, the AM fungal community composition differed between maize plants. Additionally, a greater insight into the mechanisms underlying the spatial scaling of AM fungal communities could be gained from measurements of the various soil environmental parameters which are known to contribute to the environmental heterogeneity as perceived by AM fungi, at a range of spatial scales. This would provide valuable data regarding the manner in which AM fungal niche scales through space.

#### **2.6.6 Conclusions**

Aboveground spatial patterning of host plants differs between the ecological guilds (grasses and forbs) in this study but not between species within guilds. This difference seems to drive differences in the way the associated AM fungal communities scale through space, but not in the expected manner. The aboveground spatial patterns of the grasses were more negatively spatially

autocorrelated than the forbs, yet they hosted fungal communities that were more species-rich and more grass plots displayed significant distance decay in AM fungal community similarity. The forbs were largely positively spatially autocorrelated in their aboveground patterns, yet this did not result in a greater accumulation of AM fungal species through space. It is likely that negative autocorrelation in the spatial patterns of host plants facilitates greater environmental heterogeneity that is perceptible by and influential on the associated AM fungal communities. Differences in the spatial scaling of AM fungal communities associated with the different host plant species render the detection of host plant specificity highly dependent on spatial scale of sampling.

## **Chapter 3: Spatial dependence of arbuscular mycorrhizal fungal network properties**

### **3.1 Summary**

- The analysis of AM fungal metacommunity network properties can reveal a great deal of information about the mechanisms and processes underlying their structure. However, these properties are influenced by spatial scale and depth of sampling, and by the type of metric (qualitative or quantitative) used in their analysis.
- AM fungal metacommunities associated with eight host plant species at two depths were harvested and sequencing using 454-pyrosequencing, and properties of their networks, including nestedness and dominance structure, were analysed.
- Network analysis revealed that: (1) more species-poor communities were more uneven, a pattern suggestive of a positive feedback mechanism affording a growth advantage to the earlier root colonisers; (2) there was no correlation between AM fungal occupancy and abundance, indicative of a trade-off in dispersal ability; (3) deterministic processes influence AM fungal metacommunities to a greater degree than stochastic processes; (4) observed nestedness, dominance structure and the relative influence of site quality and AM fungal phenotype on community structure are dependent on spatial scale and metric used to analyse the community.

### **3.2 Introduction**

#### *3.2.1 Nestedness in metacommunities*



A major aim in ecology is the detection and description of species distribution patterns. Such patterns, when common to multiple ecological systems, are assumed to be evidence for the action of similar processes and mechanisms. One such pattern relating to the distribution of species in metacommunities is the nested subset pattern (Wright *et al.*, 1997, Ulrich and Gotelli, 2007). While no formal mathematical definition of nestedness exists, it is commonly semantically defined as occurring in a metacommunity where the species assemblages in the most species-poor communities tend to be proper subsets of the more species-rich assemblages. Such a network property has implications for the coexistence of species and resultant stability of communities (Bascompte and Jordano, 2007). For instance, greater nestedness of interaction networks indicates functional redundancy and a greater potential for system persistence if some interactions are lost. Similarly, in metacommunities, species within a nested structure are better protected against extinction, because if one species becomes extirpated in the species-poor communities, it is more likely to have surviving populations in the more species-rich communities.

### *3.2.2 Nestedness in AM fungi*

Given the ecological and economic importance of AM fungi, and the potential insight into the processes and mechanisms underlying their spatial distribution, it is surprising that nestedness analysis has rarely been applied to AM fungal communities. When nestedness metrics have been used to study AM fungal networks, they have been used to investigate tolerance of AM fungi to soil disturbance (Kawahara and Ezawa, 2013), the effect on AM fungal communities of agricultural Management practice (Verbruggen *et al.*, 2012) and the relative roles of niche and neutral processes in structuring AM fungal communities (Chagnon *et al.*,

2012). The nestedness metrics used in these studies vary and are, with one exception (Kawahara and Ezawa, 2013), based only on qualitative matrices. These studies also vary in whether the networks are based on interaction networks, in which the two levels of the bipartite network represent the two component partners of a mutualistic partnership, or occurrence networks, in which the two levels are the species in the metacommunity and the sites in which they occur.

To test for differences in AM fungal diversity dependent on the primer pair that is used in the PCR amplification of samples, the primers ITS1 and ITS 2 were used in this study. This primer pair has a broad range of compatibility with fungi while excluding plant DNA, and can detect AM fungal taxa that AM1 and NS31 cannot. Hence this pair was used to test for the “missing diversity” that was not detected by AM1 and NS31 and to test for resultant differences in conclusions drawn about AM fungal community diversity, structure and composition.

### *3.2.3 Nestedness metrics*

Perfect nestedness occurs when every community contains a subset of those species present in the richer communities (Almeida-Neto and Ulrich, 2011). This can be demonstrated when a presence-absence species occurrence matrix is maximally packed, such that marginal totals of species presences decrease from left to right (columns) and from top to bottom (rows) (Fig. 3.1). Row totals equate to species richness per site and column totals equate to site occupancy per species.

		Species					Total
		A	B	C	D	E	
Sites	1	1	1	1	1	1	5
	2	1	1	1	1	0	4
	3	1	1	1	0	0	3
	4	1	1	0	0	0	2
	5	1	0	0	0	0	1
Total		5	4	3	2	1	

Figure 3.1. Maximally packed incidence matrix displaying perfect nestedness. 1's indicate the presence of a species, 0's indicate an absence. Column totals indicate site occupancy per species and row totals indicate species richness per site.

The two key properties accounted for in nestedness metrics using presence-absence matrices (Almeida-Neto and Ulrich, 2011):

1. Differences between column totals and between row totals.
2. Maximum overlap of non-empty cells.

The first of these equates to maximum heterogeneity in occupancy per site where each column is a species, and maximum heterogeneity in species richness where each row is a site. In an incidence matrix where each row is a site and each column is a species, as in Fig. 3.1, the second property above equates to maximum possible similarity between sites in community composition given the maximum difference in species richness and maximum degree of co-occurrence between species given maximum difference in site occupancy (Almeida-Neto and Ulrich, 2011). A pattern of perfect nestedness (Fig. 3.1), therefore, indicates a direct, positive correlation between site occupancy and species abundance. This could relate to interspecific differences within a metacommunity in the intrinsic rate of increase (Holt *et al.*, 1997).

The concept and measurement of nestedness was originally based on species incidence. Metrics such as  $T$ , the Matrix “temperature” (Atmar and Patterson, 1993), have been important to the development of our understanding of nestedness and its ecological meaning.  $T$  measures asymmetry in the incidence matrix by summarising the distribution of unexpected absences and presences either side of the boundary line representing perfect nestedness (Fig. 3.2). The matrix temperature concept has been a powerful tool to summarise metacommunities in terms of how far they deviate from perfect nestedness and thus estimate properties such as the order in which species may become extinct, relative stability of the component communities and historical habitat fluctuations (Williams and Pearson, 1997, Kolozsvary and Swihart, 1999). A greater nestedness generally means that species within the metacommunity are more resilient against extinction, as there is a greater probability that if one species is extirpated, there are populations of that species in the other communities.

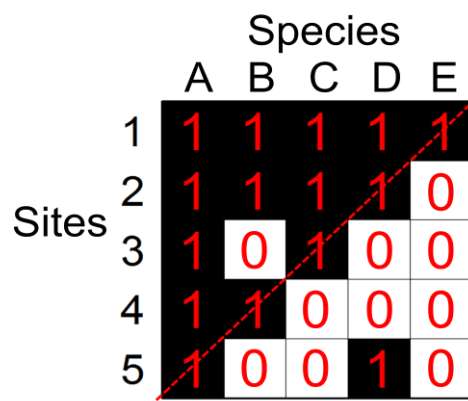


Figure 3.2. Maximally packed incidence matrix with one unexpected absence to the left of the boundary line of perfect nestedness and one unexpected presence to the right of the boundary line. Red dotted line is the boundary line of perfect nestedness. 1's indicate the presence of a species, 0's indicate an absence.

Responding to the need for a nestedness metric to correctly quantify the two matrix properties inherent to the concept of nestedness stated above, namely differences in

marginal totals and maximum overlap in presences, Almeida-Neto *et al.* (2008) developed a metric taking these properties into account. The Nestedness metric based on Overlap and Decreasing Fill (NODF) calculates the two properties decreasing fill (DF) and paired overlap (PO) using the differential proportions of 1's and 0's between columns and rows.

Using only presence-absence data, however, both of these methods fails to include species abundance data in the measure of nestedness. As such, much ecologically important information is ignored. Almeida-Neto and Ulrich (2011) devised a method to quantify nestedness using quantitative matrices instead of only presence-absence data. In addition to the two properties accounted for in presence-absence nestedness metrics above, a third property needs to be fulfilled in order to be perfectly nested according to the “Weighted Nestedness metric based on Overlap and Decreasing Fill” (WNODF) metric. Each 2 x 2 submatrix needs to be of the form:  $\begin{pmatrix} a & b \\ c & d \end{pmatrix}$ ;  $a > b, a > c, d < c, d < b$  (Fig. 3.3). The species occurrence matrix, with the added information regarding species abundances, is therefore maximally packed according to column and row totals, and then further sorted according to abundance totals (Almeida-Neto and Ulrich, 2011). The calculation of the WNODF index is as follows: in a matrix with  $n$  columns and  $m$  rows,  $F$  is the number of cells with non-zero values for any column  $c_i$  or row  $r_i$ , where  $i$  denotes column or row position and  $c_1$  is the column furthest to the left and  $r_1$  is the uppermost row. The paired nestedness between any pair of columns  $c_i$  and  $c_j$  ( $j > i$ ) will be greater than zero if  $F(c_i) > F(c_j)$ . The paired nestedness will be zero for any pair of columns where  $F(c_i) \leq F(c_j)$ . The weighted value of paired nestedness for any pair of columns with a nestedness greater than zero is then calculated as the percentage of cells in  $c_j$  that

have lower values than cells of the same row in  $c_j$ . The mean WNODF value for all pairs of columns is then calculated as: (Almeida-Neto and Ulrich, 2011)

equation 1:

$$\text{WNODFc} = 100 \sum_{i=1}^{n-1} \sum_{j=i+1}^n \frac{k_{ij}}{N_j}$$

Where  $k_{ij}$  is the number of cells with lower values in  $c_j$  and  $N_j$  is the total number of cells with non-zero values in  $c_j$ . The same procedure is used to calculate weighted values of paired nestedness for every pair of rows in the matrix. The mean paired nestedness for the  $n(n-1)/2$  pairs of columns and the  $m(m-1)/2$  pairs of rows is then calculated and used in the calculation of WNODF for the entire matrix:

equation 2:

$$\text{WNODF} = \frac{2(\text{WNODFc} + \text{WNODFr})}{n(n-1) + m(m-1)}$$

A WNODF value of 100, therefore, indicates perfect nestedness, and a value of 0 indicates no nestedness.

		Species					Total
		A	B	C	D	E	
Sites	1	5	4	3	2	1	15
	2	4	3	2	1	0	10
	3	3	2	1	0	0	6
	4	2	1	0	0	0	3
	5	1	0	0	0	0	1
Total		15	10	6	3	1	

Figure 3.3. Maximally packed matrix displaying perfect nestedness as calculated by WNODF. 1's indicate the presence of a species, 0's indicate an absence. Column totals indicate site occupancy per species and row totals indicate species richness per site.

#### 3.2.4 Advantages of a quantitative nestedness metric

Compared to the qualitative metrics  $T$ , the Matrix temperature, and NODF, the extra information contained in matrices analysed using the quantitative metric, WNODF, provides a finer-scale summary of the patterns of species abundance and distribution in metacommunities. This can result in greater insight into the processes and mechanisms underlying these patterns. Indeed, the process of sorting the quantitative species occurrence matrix, in order to maximally pack it for nestedness analysis, can itself yield much important information about the metacommunity and the component communities and species. For example, in a matrix in which columns are species, the further left a species occurs after the matrix is sorted, the greater the number of sites it occupies, and the greater its relative abundance in the entire metacommunity. Species that are more widespread and more common are therefore further to the left in sorted matrices. The order of species from left to right in sorted matrices can indicate the action of certain processes, such as inherent differences in the vital rates of the species providing an advantage to the most common species, or the effect of some random variable or variables providing a stochastically acquired advantage. If the order of species from left to right is conserved between

metacommunities, the former is more likely to be the case, whereas if the species order is idiosyncratic to each matrix, this provides evidence for stochasticity in community structuring. Similarly, the order of sites from top to bottom in a sorted matrix can indicate which sites have a greater species richness and diversity, as well a greater carrying capacity for the study taxon. Such knowledge can subsequently be related to properties of the sites that putatively facilitate larger and more diverse communities. The fact that the nestedness metric WNODF can be subdivided into its two component calculations, nestedness by rows and nestedness by columns, also provides an opportunity to infer mechanisms underlying the observed patterns. The WNODF value for the matrix in Fig. 3.4 is 50, calculated as the mean of a value of nestedness by columns of 100 (perfectly nested) and nestedness by rows of 0 (not nested). Conversely, the matrix in Fig. 3.5 has perfect nestedness among rows and no nestedness among columns, again yielding a WNODF value of 50. As can be seen from these simulated examples, the matrix perfectly nested among columns (Fig. 3.4) has a greater gradient in column totals than in row totals, signifying that the difference between the relative abundances of species in the metacommunity is greater than the difference between numbers of individuals per site across the entire metacommunity. This suggests that differences between the species in suitability to the habitat encompassed by the metacommunity, or in vital rates (e.g. fecundity, growth rate, dispersal ability) influence the observed pattern to a greater degree than differences in quality between the sites. Conversely, the simulated matrix in Fig. 3.5 is an example of a community in which the differences between numbers of individuals per site is greater than the differences between relative abundances of species in the entire metacommunity. Hence it can be inferred that the patterns observed in the metacommunity represented by the matrix in Fig. 3.5 are driven by



differences in site quality to a greater degree than by differences between the species. Absolute values of nestedness among columns and rows and the qualitative and quantitative difference between them can therefore reveal the mechanisms driving the patterns of species occurrence and abundance observed in the metacommunity. Additionally, WNODF can determine whether or not the nested pattern observed in a presence-absence matrix is consistent with a pattern in which the populations of species in more species-poor communities are smaller than their conspecific populations in richer communities (Almeida-Neto and Ulrich, 2011). If the matrix is significantly nested according to WNODF, this could be a result of a progressive loss or gain in population size across the sites. If the matrix is significantly nested according to NODF, but not according to WNODF, it can indicate: (a) that there is greater unevenness in less species-rich communities and (b) that the occupancy of a particular AM fungal OTU is not strongly positively correlated with its total abundance in the entire metacommunity. The mechanisms underlying such variation in population size could also be determined if the population size of each species in each site is compared to an external factor, such as site quality or area. Given its potential for unprecedented insight into the processes and mechanisms underlying observed patterns of community diversity, composition and structure, the benefits of using the WNODF metric for nestedness analysis in many important taxa are clear.

		Species					Total
		A	B	C	D	E	
Sites	1	5	4	3	2	1	15
	2	5	4	3	2	0	14
	3	5	4	3	0	0	12
	4	5	4	0	0	0	9
	5	5	0	0	0	0	5
Total		25	16	9	4	1	

Figure 3.4. Maximally packed matrix displaying perfect nestedness among columns as calculated by the Weighted Nestedness metric based on Overlap and Decreasing Fill (WNODF). Column totals indicate site occupancy per species and row totals indicate species richness per site.

		Species					Total
		A	B	C	D	E	
Sites	1	5	5	5	5	5	25
	2	4	4	4	4	0	16
	3	3	3	3	0	0	9
	4	2	2	0	0	0	4
	5	1	0	0	0	0	1
Total		15	14	12	9	5	

Figure 3.5. Maximally packed matrix displaying perfect nestedness among rows as calculated by Weighted Nestedness metric based on Overlap and Decreasing Fill (WNODF). Column totals indicate site occupancy per species and row totals indicate species richness per site.

### 3.2.5 Spatial dependence of network structure

Given that perceived patterns of community diversity, composition, structure and productivity are affected by spatial scale of sampling (Chase and Leibold, 2002, Keil *et al.*, 2011), it is reasonable to assume that network properties, such as nestedness and modularity, are also dependent on spatial scale. Given the implications of

particular patterns in network structure, therefore, conclusions about the relationship between occupancy and abundance (hence the prevalence of phenotypic trade-offs between AM fungal species), about the relationship between species richness and unevenness and about the relative importance of vital rates and site quality in AM fungal community structure could be highly dependent on the spatial scale of sampling. The modularity of a network is the tendency for species to be grouped into compartments or 'modules' within a network, within which interactions are more frequent than with the rest of the community. In fact, sometimes compartments can be defined as groups of elements in a network, visualised as a matrix, whose component elements do not interact at all with elements outside the group. Depending on whether the matrix represents an interaction network (of a mutualism) or an occurrence network, the presence of compartments in the network indicates either partner selectivity or endemism to a particular site or sites, respectively. The modularity and nestedness of networks can therefore inform decisions about conservation of rare, endemic or specialist species within a metacommunity. A deeper understanding of how such informative network properties in a system as important as plant-AM fungal communities are dependent upon the spatial scale of sampling and the sampling method is crucial.

### **3.3 Aims and hypotheses**

In this study the following hypotheses are being tested:

1. The detection of nestedness in AM fungal communities depends on the spatial scale of sampling, depth of sampling and the metric used.
2. AM fungal communities are nested using qualitative, but not quantitative nestedness metrics, signifying greater unevenness in species-poor

communities and a lack of correlation between AM fungal occupancy and abundance.

3. The perceived dominance structure of AM fungal communities is dependent on spatial scale of sampling.
4. The dominance structure of AM fungal OTUs is largely dependent on niche-based processes, with a negligible effect of neutral processes. The differential effects of niche and neutral processes on the dominance structure of AM fungal OTUs is dependent on spatial scale of sampling.
5. The spatial scale and depth of sampling affects the qualitative and quantitative differences between row and column nestedness and thus the conclusions drawn about whether differences between sites or differences between species have a greater influence on the observed patterns in the metacommunity.

Aims:

1. To determine the relative influence of stochastic and deterministic processes
2. To determine whether network properties are dependent on spatial scale of sampling
3. To test for evidence of phenotypic trade-off and priority effects in AM fungi

### **3.4 Materials and methods**

#### *3.4.1 Study site*

In order to quantify the effects of spatial scale and sampling depth on AM fungal community network structure, samples from an existing biodiversity experiment that was set up in 2000 were studied (Van Ruijven and Berendse, 2003, Van Ruijven and Berendse, 2009). For full details of experimental design see chapter 2. In the current

study, four monoculture plots for each of the eight plant species *Agrostis capillaris*, *Anthoxanthum odoratum*, *Festuca rubra*, *Holcus lanatus*, *Centaurea jacea*, *Leucanthemum vulgare*, *Plantago lanceolata* and *Rumex acetosa* were used. The distance between blocks was two metres, and the plots within them were one metre apart (Figs. 3.6 & 3.7). The spatial relationships between plots used in this study are outlined in Table 3.1. Two soil cores (30mm diameter, 50cm depth), 30 cm apart, were taken from the south-west quadrant of each plot in summer 2011. The soil cores were divided into sections, 2 of which were used in the current study; from 0 - 5cm in depth, hereafter referred to as depth 1, and from 20 - 35cm, hereafter referred to as depth 4. Approximately 100 mg of fresh root material was stored at - 80°C for molecular analyses. In order to amass sufficient biomass for DNA extraction, the two cores were pooled for each plot, after having been separated into sections of different depths. For plots in which the root biomass in the south-west quadrant was too low for harvest, alternative quadrants were used for sampling. In block 1, the south-east and the north-west quadrants were used for the *Rumex* and *Holcus* plots respectively. In block 2, the south-east and north-east quadrants were used for the *Plantago* and *Rumex* plots respectively. In block 3, the north-west quadrant was used for *Anthoxanthum*. In block 4, the north-west corner was used for *Leucanthemum*, and in block 5, the north-east quadrant was used in the sampling of the *Agrostis* plot. For the analysis of the dominance structure of AM fungi, the communities in the four replicate plots per plant species were compared. For the nestedness analysis, the data from the four plots were pooled and the communities were analysed according to sampling depth and the related differences in spatial scale.

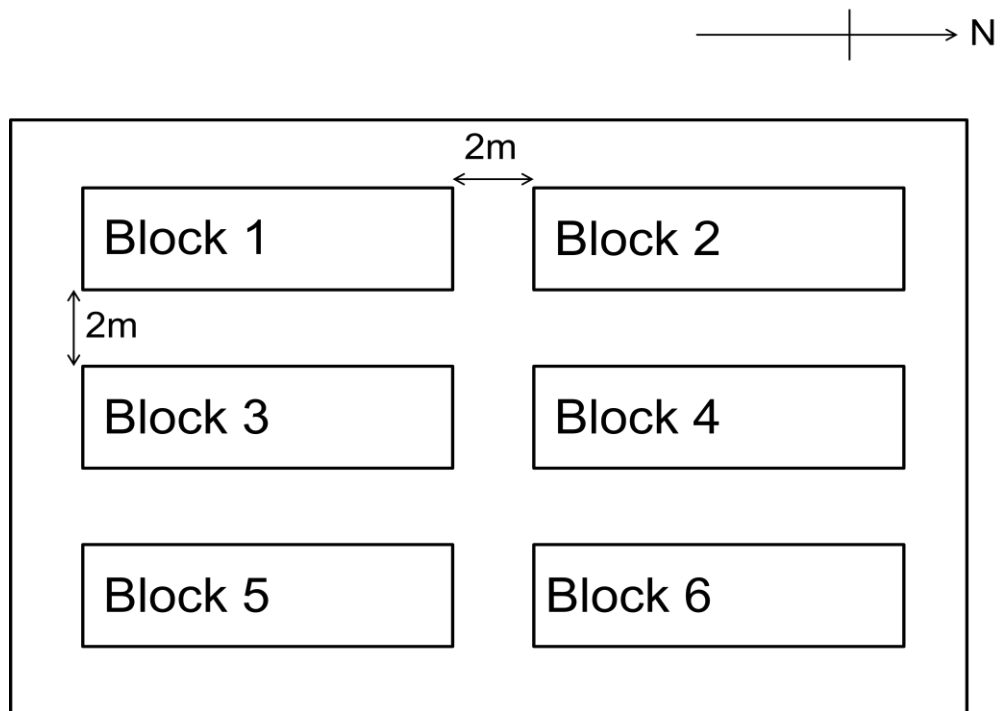


Figure 3.6. Spatial arrangement of blocks within each of which 18 experimental plots were established.

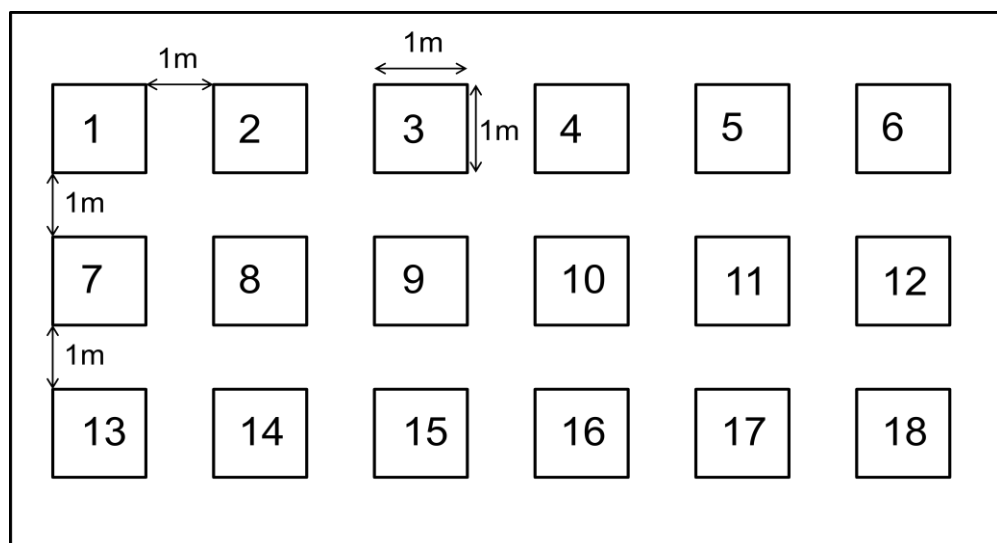


Figure 3.7. Spatial arrangement of plots within each block (not to scale)

Table 3.1. Positions of host plant monocultures plots of each species within each block. See Figs. 3.6 & 3.7.

Plant species	Block					
	1	2	3	4	5	6
<i>A. capillaris</i>	7	7	12	17	7	10
<i>A. odoratum</i>	5	13	2	14	3	2
<i>F. rubra</i>	17	4	11	3	5	6
<i>H. lanatus</i>	16	11	8	2	1	12
<i>C. jacea</i>	14	15	16	1	17	13
<i>L. vulgare</i>	18	8	5	15	11	11
<i>P. lanceolata</i>	1	2	14	8	10	9
<i>R. acetosa</i>	13	18	7	12	16	14

### 3.4.2 Molecular methods

DNA extraction, amplification and sequencing was carried out by Anne Cotton at the University of Wageningen and sequence denoising, clustering and taxonomy assignment done by Alex Dumbrell at the University of Essex.

#### 3.4.2.1 DNA extraction

DNA was extracted from the root samples using DNeasy Plant mini kit (Qiagen Ltd, W Sussex, UK) according to the manufacturer's instructions.

#### 3.4.2.2 DNA amplification and 454 GS FLX pyrosequencing of amplicon libraries

The internal transcribed spacer (ITS1) region of ribosomal DNA was amplified by PCR using GoTaq Flexi DNA Polymerase (Promega, Leiden, the Netherlands) and the primers ITSF1 and ITS2 (Buee *et al.*, 2009). The ITS1 region was sequenced using the 454 GS-FLX XLR70 plate separated with a two-lane gasket (454 Life

Sciences/Roche Applied Biosystems, Nutley, NJ, USA) from the ITS1F end at Plant Research International, Wageningen UR, Wageningen, The Netherlands.

### 3.4.3 Data analysis

#### 3.4.3.1 Sequence analysis

Pyrosequence reads were analysed using the QIIME pipeline and its associated modules (Caporaso *et al.*, 2010). The flowgram denoiser algorithm (Reeder and Knight, 2010) was used to fully denoise the pyrosequencing data. All sequences were checked for the presence of correct pyrosequencing adaptors, 10-bp barcodes and taxon-specific primers and those containing errors in these regions were removed. Sequences <200bp in length, those with low quality scores (<25), and sequences containing homopolymer inserts were also removed. The USEARCH algorithm (Edgar, 2010) was used to cluster pyrosequence reads into operational taxonomic units (OTUs), defined by sequence identity within OTUs of greater than 97%, and the UCHIME de novo chimera detection program (Edgar *et al.*, 2011) was used to detect and remove all chimeras and any OTU represented by fewer than four sequences. Sequence taxonomy was assigned using BLAST against NCBI. 11672 sequences were Glomeromycotan.

To determine the likelihood that increased sampling effort would qualitatively affect the results, species accumulation curves using individual-based rarefaction were computed using these sequences. Unless otherwise indicated all analyses were conducted in the R statistical language using the vegan and bipartite libraries (R Development Core Team, 2011).



### 3.4.3.2 Network analyses

In qualitative analyses of the network, when at least a single read was detected for an OTU within a sample, a presence was recorded; otherwise, an absence was recorded. In quantitative analyses, in which AM fungal OTU abundance, as opposed to only presence or absence, is taken into account, the number of reads for each OTU was used as a proxy for the relative abundance of the OTU within each sample.

#### 3.4.3.2.1 Qualitative network analysis

Compartments within a network are subsets of the network which are not connected to another compartment, through either of the levels within a bipartite network. The compartment diversity is the Shannon diversity index of the compartments, taking into account their number and size (Tylianakis *et al.*, 2007). The NODF (Nestedness based on Overlap and Decreasing Fill) metric calculates the combined degree to which marginal totals differ, and the degree to which filled cells in rows and columns with lower marginal totals are subsets of those in rows and columns with greater marginal totals (Almeida-Neto *et al.*, 2008). NODF ranges from 0 (no nestedness) to 100 (perfectly nested). In addition to a metric for the whole matrix, NODF is partitioned into nestedness by columns and nestedness by rows. The significance of the three NODF metrics were evaluated using the null model simulation method “r2dtable” in the vegan library, which keeps the row, column and matrix sums constant. 99 null communities were simulated to test the observed community for significance.

#### *3.4.3.2.2 Quantitative network analysis*

The WNODF metric uses the abundance data (Number of reads per AM fungal species at each site) to determine the weighted nestedness for the entire matrix, and separately for rows and for columns. To test whether the nested pattern observed in the quantitative matrices were consistent with a pattern in which the populations of species in more species-poor communities are smaller than their conspecific populations in richer communities, the difference between the NODF and the WNODF was calculated for each host plant species at each depth and scale. The AM fungal species richness was also determined for each network.

### **3.5 Results**

#### **3.5.1 Primer-dependent differences in AM fungal richness**

In the study in Chapter 2, in which the AM1-NS31 primer pair was used to profile the AM fungal community, 15 AM fungal taxa were recorded from the 12 monoculture plots of four plant species. In this study, in which the primer pair ITS1-ITS2 was used, 85 AMF OTUs were recorded from the 32 monoculture plots of eight plant species.

#### **3.5.2 Community diversity, structure and spatial scale**

##### **3.5.2.1 Species accumulation by species and by depth**

Eighty-five AMF OTUs were detected from the eight host plant species in monoculture combined. Species accumulation curves were computed using rarefaction. The rarefied species accumulation curve for all monoculture plots

combined reached an asymptote (Fig. 3.8) and thus, further sampling was likely to have made no qualitative difference to the results. The species accumulation curves for each host plant species (Fig. 3.9) reveals the varying rates at which AMF OTUs are accumulated through space for each of the eight host plant species, as well as the differences in total species richness. Similarly, the rate of species accumulation differs between depths. The later asymptote and lower total species richness in the shallower depth reveals the difference between the two depths in AMF community species richness.

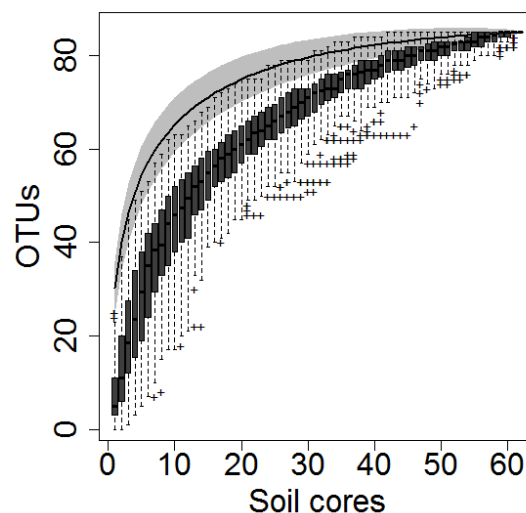


Figure 3.8. AM fungal species (OTU) accumulation curve (light grey envelope) using individual-based rarefaction on 454-pyrosequence data for all monoculture plots of all host plant species (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Festuca rubra*, *Holcus lanatus*, *Centaurea jacea*, *Leucanthemum vulgare*, *Plantago lanceolata* and *Rumex acetosa*) at both depths. Boxplots display the mean and standard deviation of species richness per soil core.

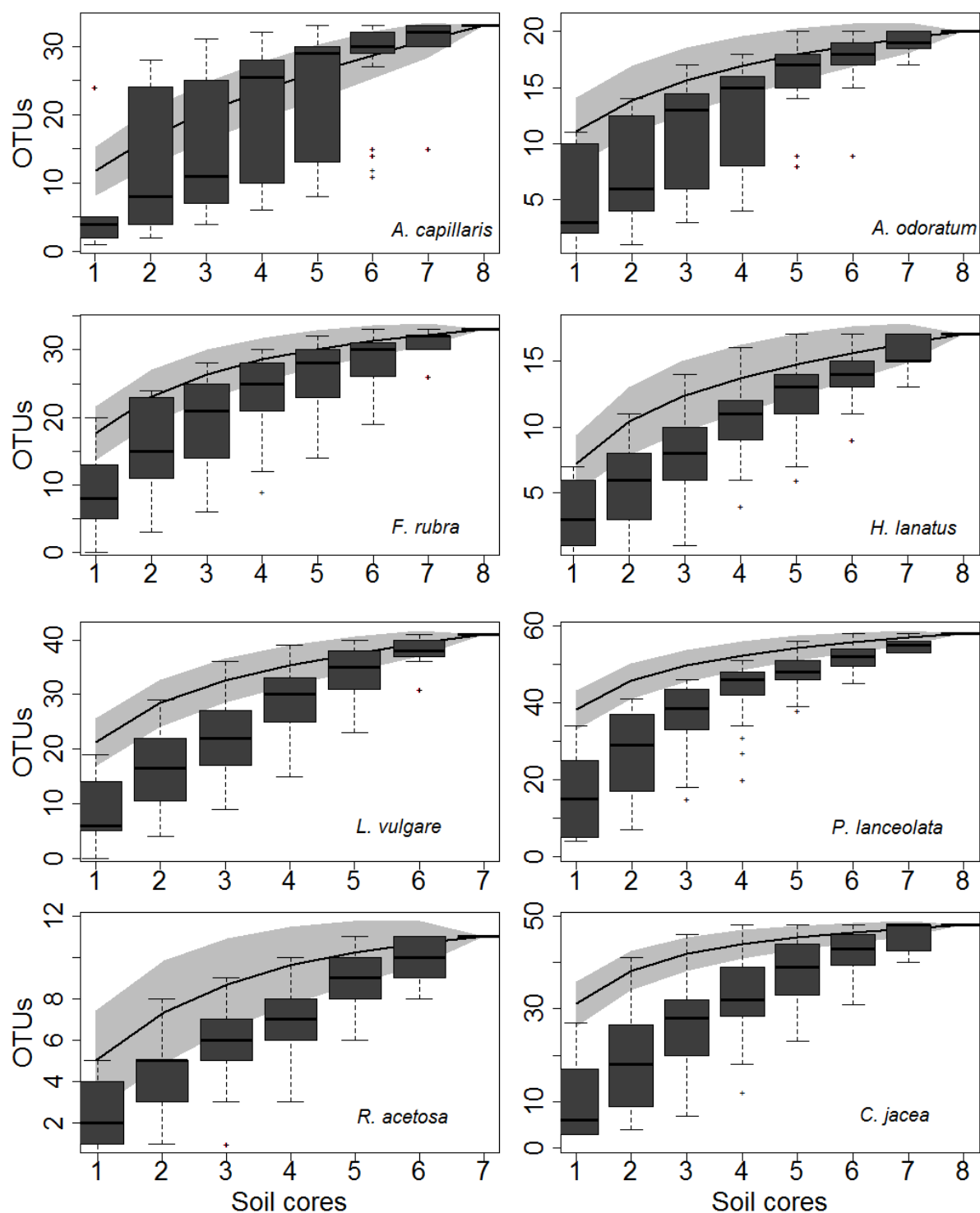


Figure 3.9. Species accumulation curves (light grey envelopes) using individual-based rarefaction on 454-pyrosequence data from all four plots and both depths for each of the eight host plant species separately (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Festuca rubra*, *Holcus lanatus*, *Centaurea jacea*, *Leucanthemum vulgare*, *Plantago lanceolata* and *Rumex acetosa*). Boxplots display the mean and standard deviation of species richness per soil core.

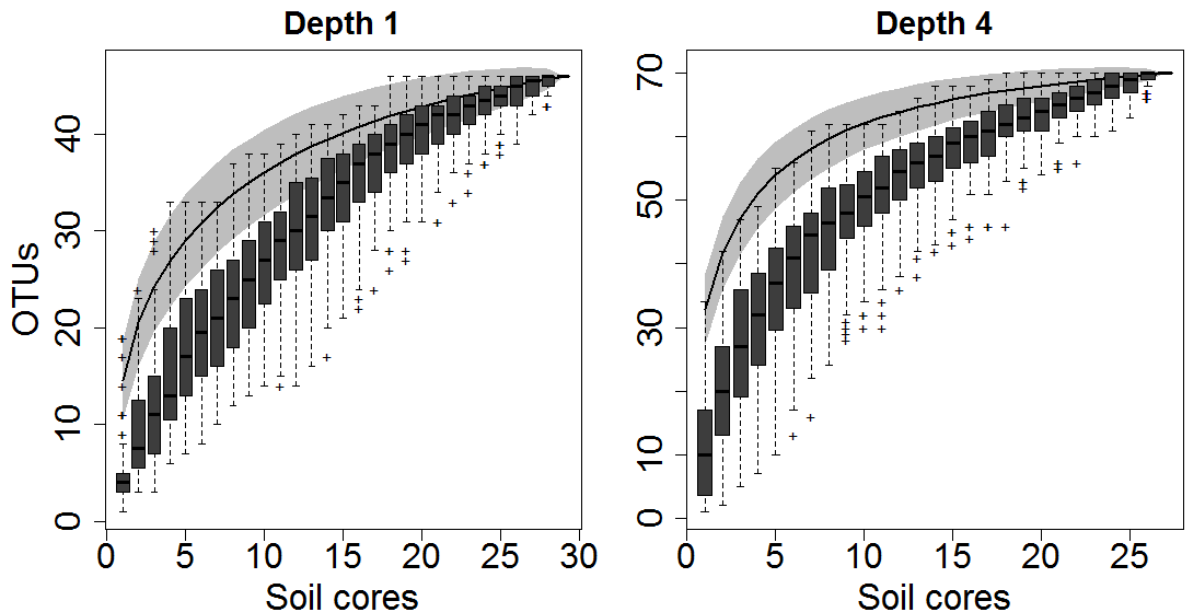


Figure 3.10. Species accumulation curves (light grey envelopes) for all monoculture plots and all eight host plant species, at depth 1 and depth 4 separately. Boxplots display the mean and standard deviation of species richness per soil core.

### 3.5.3 Determination of the most abundant AMF OTU in the community depends on spatial scale

The most abundant OTU in each of the four plots is not always the same OTU that is most abundant when the four plots are pooled (Figs. 3.11 - 3.18). The most abundant AM fungal OTU when data from all four plots per plant species were pooled was also the most abundant in only one of the four replicate plots in all host plant species apart from *A. odoratum*. The most abundant OTU in the four *A. odoratum* plots combined was also the most abundant OTU in two of the four replicate plots.

### 3.5.4 Dominance structure

AM fungal OTU 61 was the most abundant OTU in four of the eight host plant species (*A. capillaris*, *A. odoratum*, *H. lanatus* and *C. jacea*), when the data from all four replicate plots per species was pooled. OTU 83 was most abundant in three plots (*F. rubra*, *L. vulgare* and *R. acetosa*) and OTU 90 was most abundant in one

plot (*P. lanceolata*). Twelve AM fungal OTUs were represented in the three most abundant OTUs per host plant species when the data from all four replicate plots was taken into account (Table 3.2). The four most abundant OTUs overall were the same for both grasses and forbs, but the other eight OTUs represented in the three most abundant OTUs differed as to whether they occurred in grass species or in forb species. OTU 61 was the most abundant OTU in 31% of communities, and OTU 83 was the most abundant OTU in 29% of communities. OTU 90 was the most abundant OTU in 8.6% of all communities profiled (Table 3.3).

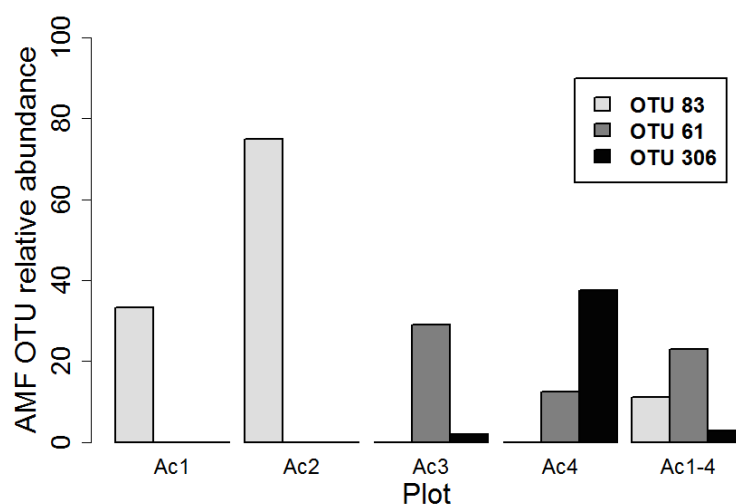


Figure 3.11. The relative abundance of the three most abundant AM fungal OTUs in the four *A. capillaris* plots (Ac1-4), separately and combined.

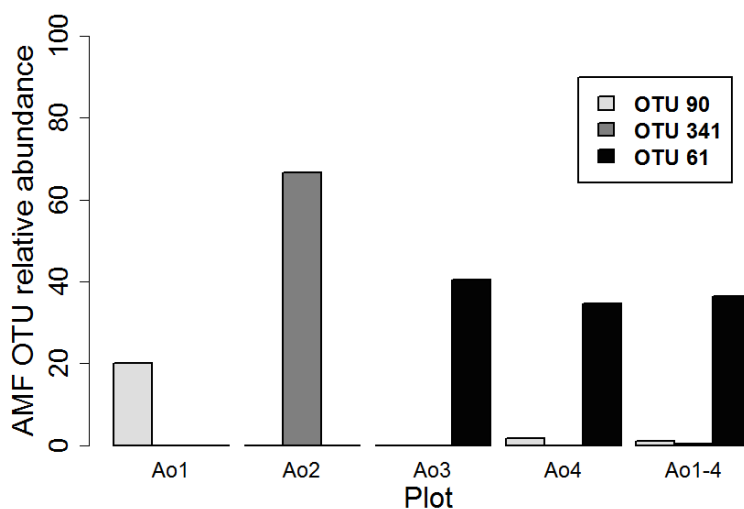


Figure 3.12. The relative abundance of the three most abundant AM fungal OTUs in the four *A. odoratum* plots (Ao1-4), separately and combined.

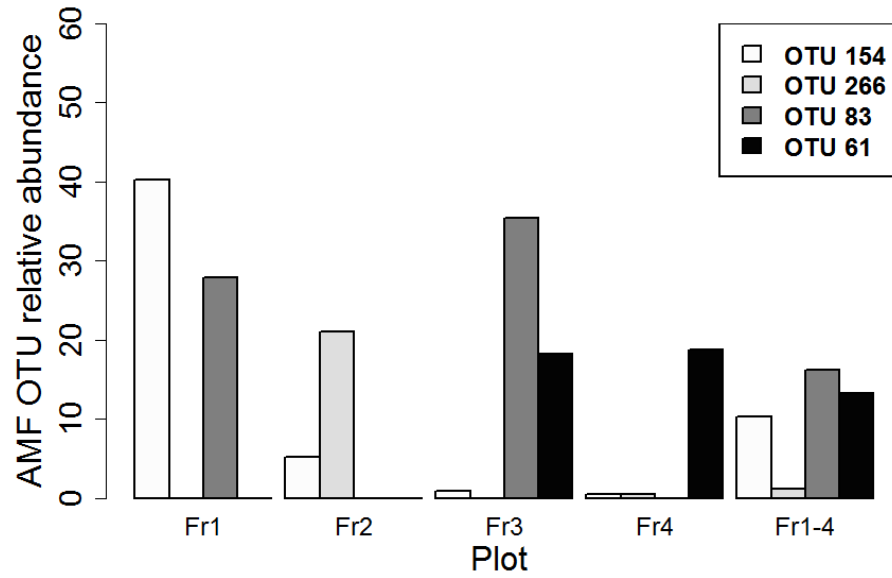


Figure 3.13. The relative abundance of the four most abundant AM fungal OTUs in the four *F. rubra* plots (Fr1-4), separately and combined.

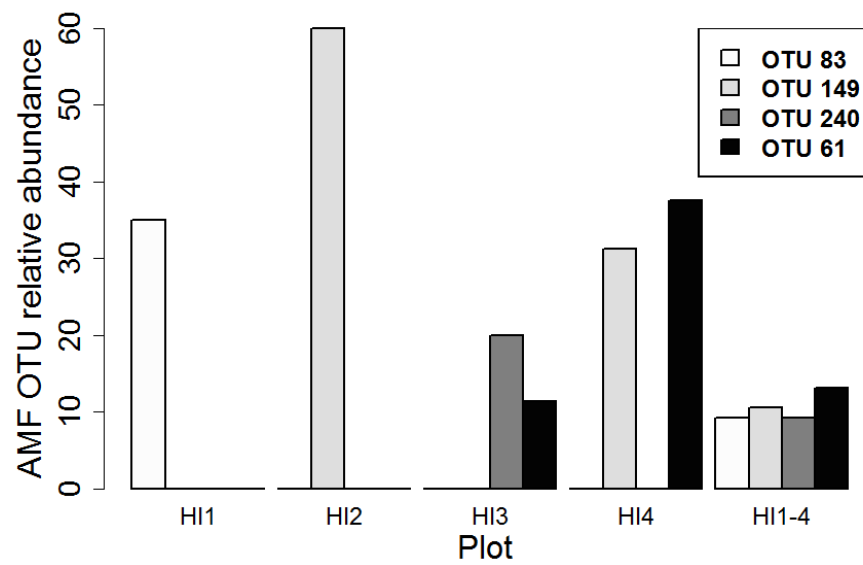


Figure 3.14. The relative abundance of the four most abundant AM fungal OTUs in the four *H. lanatus* plots (HI1-4), separately and combined.

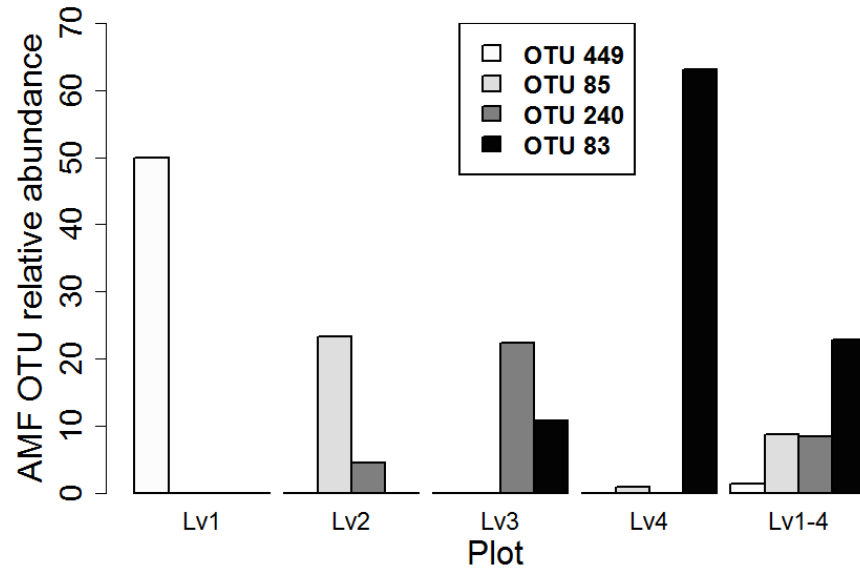


Figure 3.15. The relative abundance of the four most abundant AM fungal OTUs in the four *L. vulgare* plots (Lv1-4), separately and combined.

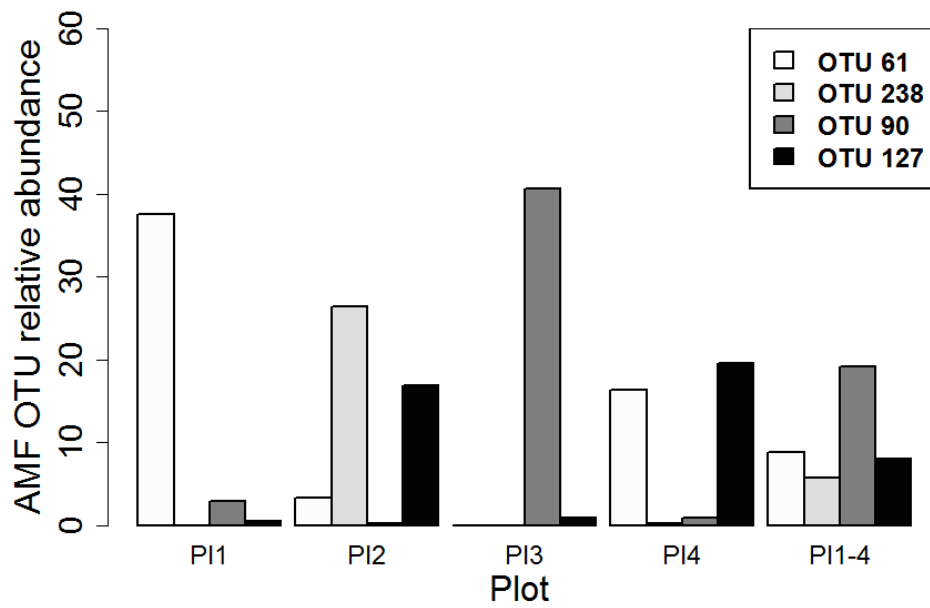


Figure 3.16. The relative abundance of the four most abundant AM fungal OTUs in the four *P. lanceolata* plots (PI1-4), separately and combined.



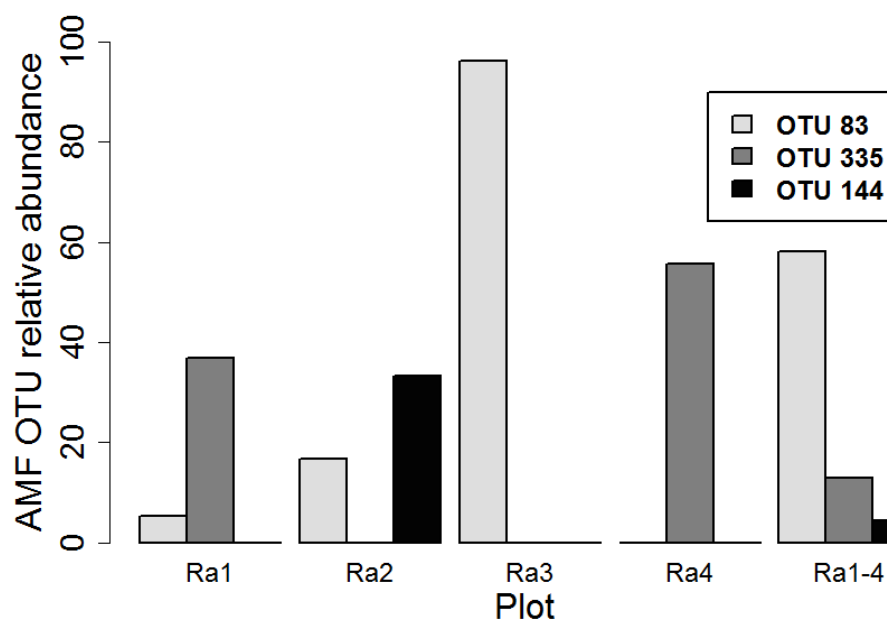


Figure 3.17. The relative abundance of the three most abundant AM fungal OTUs in the four *R. acetosa* plots (Ra1-4), separately and combined.

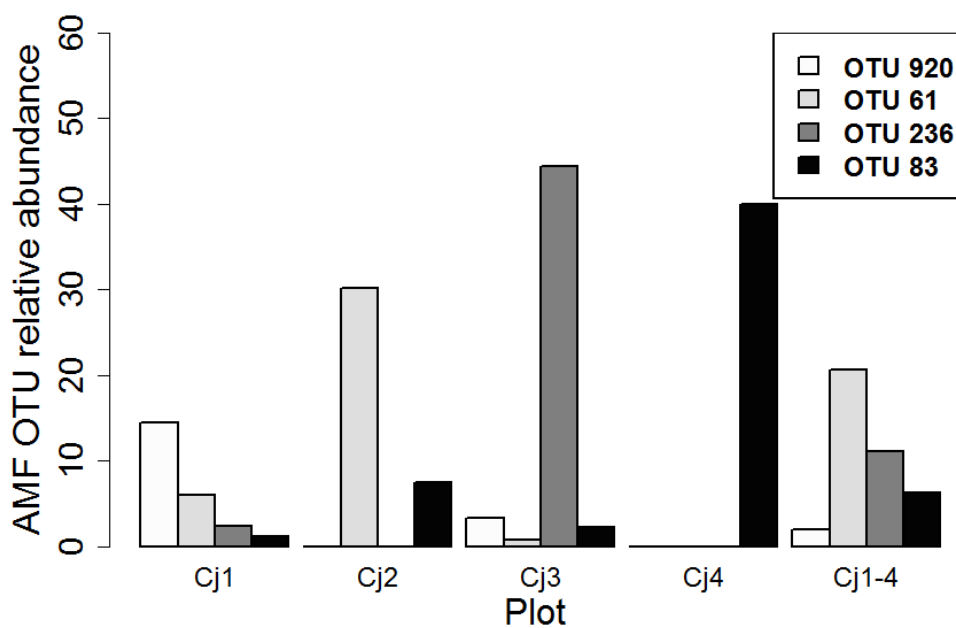


Figure 3.18. The relative abundance of the four most abundant AM fungal OTUs in the four *C. jacea* plots (Cj1-4), separately and combined.

Table 3.2. Frequency of occurrence of AM fungal OTUs in the three most abundant OTUs for the eight host plants, when data was pooled from each of the four replicate plots per plant species. Greater numbers highlighted with darker shades of orange.

	AM fungal OTU											
	83	61	90	240	306	341	154	149	127	335	144	236
Grasses	3	4	1	1	1	1	1	1	0	0	0	0
Forbs	3	2	1	1	0	0	0	0	1	1	1	1
Total	6	6	2	2	1	1	1	1	1	1	1	1

Table 3.3. Frequency of AM fungal OTU occurrence as the most abundant OTU in the community as a percentage of total number of communities, for the four replicate plots per host plant without the pooled data (Single plots) and for all plots and the combined data (All samples). A three-colour scale (green-yellow-red), ranging from green (minimum) to red (maximum) used to indicate value

	AM fungal OTU											
	83	61	90	240	306	341	154	149	127	335	144	236
Single plots	22	26	7.4	7.4	3.7	3.7	3.7	7.4	3.7	7.4	3.7	3.7
All samples	29	31	8.6	5.7	2.9	2.9	2.9	2.9	2.9	5.7	2.9	2.9

A Friedman rank sum test revealed that the rank order of AM fungal OTU abundance was significantly different between the 24 communities tested (two depths individually and both depths combined for each of the eight host plant species),  $X^2(23) = 236.17$ ,  $P < 0.001$ . This was the case both when qualitative data was used (presences and absences indicating occurrence of OTUs across all samples) and when quantitative data (frequency of 454 reads per OTU) was used. A post-hoc Nemenyi test revealed that, when quantitative data was used, *R. acetosa* was the most different in terms of rank order of OTUs, with 44% of pairwise comparisons involving *R. acetosa* displaying significant difference, followed by *A. capillaris* (35%), *H. lanatus* (32%), *A. odoratum* (30%), *L. vulgare* (23%), *C. jacea* (21%), *F. rubra* (21%) and *P. lanceolata* (Tables 3.4 & 3.5). *P. lanceolata* was the most similar, with only 15% of pairwise comparisons involving *P. lanceolata* displaying a significant difference (Table 3.5). This order was slightly different when using qualitative data,

with *R. acetosa* the most different (45%), followed by *A. capillaris* (33%), *H. lanatus* (32%), *A. odoratum* (29%), *F. rubra* (24%), *L. vulgare* (24%), *C. jacea* (21%) and *P. lanceolata* (14%) (Tables 3.6 & 3.7). There was a significant difference in the number of pairwise comparisons that were significant between host plant species using quantitative data ( $F_{7,56} = 2.62$ ,  $P = 0.02$ ), but not using qualitative data ( $F_{7,56} = 1.75$ ,  $P = 0.12$ ), and a post-hoc Tukey's multiple comparison of means procedure revealed that only the difference (using quantitative data) between *P. lanceolata* and *R. acetosa* was significant ( $P = 0.03$ ). When the quantitative data was grouped by depth, depth 1 showed the greatest difference in rank order of OTUs, with 37% of pairwise comparisons involving depth 1 displaying significant difference. 25% and 24% of pairwise comparisons involving depth 4 and depths 1 and 4 combined, respectively, displayed a significant difference. When qualitative data was used and grouped by depth, the same pattern was observed, except 35% of the pairwise comparisons involving depth 1 were significantly different in terms of AM fungal OTU rank order. None of the differences between depths 1, 4 and 1 and 4 combined in the number of pairwise comparisons that were significant were significant using either quantitative ( $F_{2,6} = 1.64$ ,  $P = 0.27$ ) or qualitative ( $F_{2,6} = 0.42$ ,  $P = 0.68$ ) data. Overall, using either quantitative or qualitative data, 28% of pairwise comparisons were significantly different in terms of AM fungal OTU rank order.

There was a significant difference in the Nemenyi statistic values between depths using quantitative data ( $F_{2,465} = 3.64$ ,  $P = 0.027$ ), in which a Tukey's procedure revealed a significant difference between depths 1 and depth 1 and 4 combined ( $P = 0.034$ ). Using qualitative matrices, there was no significant difference between Nemenyi statistic values observed ( $F_{2,465} = 2.25$ ,  $P = 0.11$ ). A significant difference in Nemenyi values was observed between host plant species both using quantitative

( $F_{7,520} = 4.92$ ,  $P < 0.001$ ) and qualitative ( $F_{7,520} = 4.72$ ,  $P < 0.001$ ) data. A Tukey's procedure revealed that three pairwise comparisons were significantly different when quantitative data was used (*R. acetosa*/*C. jacea*,  $P < 0.001$ , *R. acetosa*/*L. vulgare*,  $P < 0.01$  and *R. acetosa*/*P.lanceolata*,  $P < 0.001$ ), and that four pairwise comparisons were significantly different when qualitative data was used (*R. acetosa*/*C. jacea*,  $P < 0.001$ , *R. acetosa*/*F.rubra*,  $P = 0.03$ , *R. acetosa*/*L. vulgare*,  $P < 0.01$  and *R. acetosa*/*P.lanceolata*,  $P < 0.001$ ).

Table. 3.4. Heat map of Nemenyi test statistics for all pairwise combinations of AM fungal communities, grouped into Host plant species. Quantitative data (454-read frequency) were used in the ranking of AM fungal OTUs. A three-colour scale (blue-green-red), ranging from blue (minimum) to red (maximum) used to indicate value of test statistic.

	AcD1	AcD4	AcD1.4	AoD1	AoD4	AoD1.4	CjD1	CjD4	CjD1.4	FrD1	FrD4	FrD1.4	HID1	HID4	HID1.4	LvD1	LvD4	LvD1.4	PID1	PID4	PID1.4	RaD1	RaD4
AcD4	6.14																						
AcD1.4	8.40	2.26																					
AoD1	0.92	7.06	9.32																				
AoD4	3.35	2.78	5.05	4.27																			
AoD1.4	5.28	0.85	3.11	6.20	1.93																		
CjD1	5.97	0.17	2.43	6.89	2.62	0.68																	
CjD4	7.34	1.20	1.06	8.26	3.99	2.06	1.37																
CjD1.4	6.11	0.02	2.29	7.03	2.76	0.83	0.15	1.23															
FrD1	2.24	3.90	6.16	3.16	1.11	3.04	3.73	5.10	3.87														
FrD4	7.16	1.02	1.24	8.08	3.80	1.87	1.19	0.18	1.04	4.92													
FrD1.4	8.39	2.25	0.01	9.31	5.04	3.11	2.42	1.05	2.28	6.15	1.23												
HID1	1.86	8.00	10.26	0.94	5.22	7.15	7.83	9.20	7.98	4.10	9.02	10.25											
HID4	3.15	2.98	5.25	4.07	0.20	2.13	2.81	4.19	2.96	0.91	4.00	5.24	5.02										
HID1.4	4.92	1.21	3.47	5.84	1.57	0.36	1.04	2.42	1.19	2.68	2.23	3.47	6.79	1.77									
LvD1	6.69	0.55	1.71	7.61	3.34	1.40	0.72	0.65	0.58	4.45	0.47	1.70	8.55	3.54	1.76								
LvD4	6.14	0.01	2.25	7.06	2.79	0.86	0.18	1.20	0.03	3.90	1.01	2.25	8.01	2.99	1.22	0.54							
LvD1.4	7.17	1.04	1.23	8.09	3.82	1.89	1.20	0.17	1.06	4.93	0.02	1.22	9.03	4.02	2.25	0.48	1.03						
PID1	5.52	0.61	2.88	6.44	2.17	0.24	0.44	1.82	0.59	3.28	1.63	2.87	7.39	2.37	0.60	1.17	0.62	1.65					
PID4	4.89	1.25	3.51	5.81	1.53	0.40	1.08	2.45	1.23	2.65	2.27	3.51	6.75	1.73	0.04	1.80	1.26	2.29	0.64				
PID1.4	5.03	1.10	3.37	5.95	1.68	0.25	0.94	2.31	1.08	2.79	2.12	3.36	6.90	1.88	0.11	1.66	1.11	2.14	0.49	0.15			
RaD1	0.85	5.28	7.55	1.77	2.50	4.43	5.12	6.49	5.26	1.39	6.30	7.54	2.72	2.30	4.07	5.84	5.29	6.32	4.67	4.03	4.18		
RaD4	2.68	8.82	11.08	1.76	6.04	7.97	8.65	10.02	8.80	4.92	9.84	11.07	0.82	5.84	7.61	9.37	8.83	9.86	8.21	7.57	7.72	3.54	
RaD1.4	2.52	3.62	5.88	3.44	0.84	2.77	3.45	4.82	3.60	0.28	4.64	5.87	4.38	0.64	2.41	4.17	3.63	4.66	3.01	2.37	2.52	1.66	5.20

Table 3.5. *P* values of Nemenyi test statistics for all pairwise combinations of AM fungal communities, grouped into Host plant species. Quantitative data (454-read frequency) were used in the ranking of AM fungal OTU. Bold values, highlighted in grey indicate significance at the  $P < 0.05$  level.

	AcD1	AcD4	AcD1.4	AoD1	AoD4	AoD1.4	CjD1	CjD4	CjD1.4	FrD1	FrD4	FrD1.4	HID1	HID4	HID1.4	LvD1	LvD4	LvD1.4	PID1	PID4	PID1.4	RaD1	RaD4
AcD4	<b>0.003</b>																						
AcD1.4	<b>0.000</b>	0.997																					
AoD1	1.000	<b>0.000</b>	<b>0.000</b>																				
AoD4	0.771	0.955	0.063	0.281																			
AoD1.4	<b>0.036</b>	1.000	0.870	<b>0.003</b>	1.000																		
CjD1	<b>0.006</b>	1.000	0.991	<b>0.000</b>	0.978	1.000																	
CjD4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.421	0.999	1.000																
CjD1.4	<b>0.004</b>	1.000	0.996	<b>0.000</b>	0.959	1.000	1.000	1.000															
FrD1	0.997	0.472	<b>0.003</b>	0.853	1.000	0.893	0.568	0.055	0.485														
FrD4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.524	1.000	1.000	1.000	1.000	0.084													
FrD1.4	<b>0.000</b>	0.997	1.000	<b>0.000</b>	0.064	0.873	0.991	1.000	0.996	<b>0.003</b>	1.000												
HID1	1.000	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.042</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.361	<b>0.000</b>	<b>0.000</b>											
HID4	0.856	0.911	<b>0.039</b>	0.377	1.000	0.999	0.950	0.320	0.917	1.000	0.413	<b>0.040</b>	0.067										
HID1.4	0.082	1.000	0.709	<b>0.008</b>	1.000	1.000	1.000	0.992	1.000	0.970	0.997	0.713	<b>0.000</b>	1.000									
LvD1	<b>0.001</b>	1.000	1.000	<b>0.000</b>	0.778	1.000	1.000	1.000	1.000	0.209	1.000	1.000	<b>0.000</b>	0.676	1.000								
LvD4	<b>0.003</b>	1.000	0.997	<b>0.000</b>	0.954	1.000	1.000	1.000	1.000	0.468	1.000	0.997	<b>0.000</b>	0.909	1.000	1.000							
LvD1.4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.516	1.000	1.000	1.000	1.000	0.081	1.000	1.000	<b>0.000</b>	0.405	0.997	1.000	1.000						
PID1	<b>0.019</b>	1.000	0.937	<b>0.001</b>	0.998	1.000	1.000	1.000	1.000	0.803	1.000	0.939	<b>0.000</b>	0.993	1.000	1.000	1.000	1.000					
PID4	0.089	1.000	0.688	<b>0.009</b>	1.000	1.000	1.000	0.990	1.000	0.974	0.996	0.693	<b>0.000</b>	1.000	1.000	1.000	1.000	0.996	1.000				
PID1.4	0.065	1.000	0.763	<b>0.006</b>	1.000	1.000	1.000	0.995	1.000	0.954	0.999	0.767	<b>0.000</b>	1.000	1.000	1.000	1.000	0.998	1.000	1.000			
RaD1	1.000	<b>0.036</b>	<b>0.000</b>	1.000	0.987	0.215	0.053	<b>0.001</b>	<b>0.038</b>	1.000	<b>0.002</b>	<b>0.000</b>	0.966	0.996	0.377	<b>0.008</b>	<b>0.035</b>	<b>0.002</b>	0.139	0.397	0.323		
RaD4	0.970	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.005</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.082	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.008</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.676	
RaD1.4	0.986	0.629	<b>0.007</b>	0.729	1.000	0.958	0.721	0.102	0.642	1.000	0.147	<b>0.007</b>	0.236	1.000	0.992	0.327	0.625	0.143	0.904	0.993	0.986	1.000	<b>0.044</b>

Table. 3.6. Heat map of Nemenyi test statistics for all pairwise combinations of AM fungal communities, grouped into Host plant species. Qualitative data (no. of presences of OTU) were used in the ranking of AM fungal OTUs. A three-colour scale (blue-green-red), ranging from blue (minimum) to red (maximum) used to indicate value of test statistic.

	AcD1	AcD4	AcD1.4	AoD1	AoD4	AoD1.4	CjD1	CjD4	CjD1.4	FrD1	FrD4	FrD1.4	HID1	HID4	HID1.4	LvD1	LvD4	LvD1.4	PID1	PID4	PID1.4	RaD1	RaD4
AcD4	6.11																						
AcD1.4	8.57	2.46																					
AoD1	0.97	7.07	9.53																				
AoD4	3.44	2.67	5.13	4.40																			
AoD1.4	5.42	0.68	3.14	6.39	1.99																		
CjD1	5.80	0.31	2.77	6.76	2.36	0.38																	
CjD4	7.30	1.20	1.27	8.27	3.87	1.88	1.50																
CjD1.4	5.61	0.49	2.95	6.58	2.18	0.19	0.18	1.69															
FrD1	2.95	3.16	5.62	3.91	0.49	2.48	2.85	4.36	2.67														
FrD4	7.04	0.94	1.53	8.01	3.60	1.62	1.24	0.26	1.43	4.10													
FrD1.4	8.32	2.22	0.25	9.29	4.89	2.90	2.52	1.02	2.71	5.38	1.28												
HID1	1.88	7.98	10.45	0.91	5.32	7.30	7.68	9.18	7.49	4.82	8.92	10.20											
HID4	2.98	3.13	5.59	3.94	0.46	2.45	2.82	4.33	2.64	0.03	4.06	5.35	4.85										
HID1.4	4.72	1.39	3.85	5.68	1.28	0.71	1.08	2.58	0.90	1.77	2.32	3.60	6.60	1.74									
LvD1	7.17	1.07	1.40	8.14	3.74	1.75	1.37	0.13	1.56	4.23	0.13	1.15	9.05	4.20	2.45								
LvD4	6.07	0.03	2.49	7.04	2.64	0.65	0.28	1.23	0.46	3.13	0.97	2.25	7.95	3.10	1.36	1.10							
LvD1.4	7.36	1.25	1.21	8.32	3.92	1.93	1.56	0.05	1.74	4.41	0.31	0.97	9.23	4.38	2.64	0.18	1.28						
PID1	5.25	0.85	3.31	6.22	1.82	0.17	0.54	2.05	0.36	2.31	1.79	3.07	7.13	2.28	0.54	1.92	0.82	2.10					
PID4	4.54	1.56	4.03	5.51	1.10	0.88	1.26	2.76	1.07	1.60	2.50	3.78	6.42	1.56	0.18	2.63	1.53	2.81	0.71				
PID1.4	4.05	2.06	4.52	5.02	0.61	1.37	1.75	3.25	1.56	1.10	2.99	4.27	5.93	1.07	0.67	3.12	2.02	3.31	1.20	0.49			
RaD1	1.50	4.61	7.07	2.46	1.94	3.93	4.30	5.81	4.12	1.45	5.55	6.83	3.37	1.48	3.22	5.68	4.58	5.86	3.76	3.04	2.55		
RaD4	2.68	8.78	11.24	1.71	6.11	8.10	8.47	9.98	8.29	5.62	9.72	11.00	0.80	5.65	7.39	9.85	8.75	10.03	7.93	7.22	6.73	4.17	
RaD1.4	1.47	4.63	7.09	2.44	1.96	3.95	4.33	5.83	4.14	1.47	5.57	6.85	3.35	1.50	3.24	5.70	4.60	5.88	3.78	3.07	2.58	0.02	4.15

Table. 3.7. *P* values of Nemenyi test statistics for all pairwise combinations of AM fungal communities, grouped into Host plant species. Qualitative data (no. of presences of OTU) were used in the ranking of AM fungal OTU. Bold values, highlighted in grey indicate significance at the  $P < 0.05$  level.

	AcD1	AcD4	AcD1.4	AoD1	AoD4	AoD1.4	CjD1	CjD4	CjD1.4	FrD1	FrD4	FrD1.4	HID1	HID4	HID1.4	LvD1	LvD4	LvD1.4	PID1	PID4	PID1.4	RaD1	RaD4
AcD4	<b>0.004</b>																						
AcD1.4	<b>0.000</b>	0.989																					
AoD1	1.000	<b>0.000</b>	<b>0.000</b>																				
AoD4	0.729	0.972	0.052	0.227																			
AoD1.4	<b>0.025</b>	1.000	0.859	<b>0.002</b>	1.000																		
CjD1	<b>0.009</b>	1.000	0.958	<b>0.000</b>	0.994	1.000																	
CjD4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.489	1.000	1.000																
CjD1.4	<b>0.015</b>	1.000	0.919	<b>0.001</b>	0.998	1.000	1.000	1.000															
FrD1	0.921	0.853	<b>0.015</b>	0.464	1.000	0.988	0.942	0.245	0.972														
FrD4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.638	1.000	1.000	1.000	1.000	0.365													
FrD1.4	<b>0.000</b>	0.997	1.000	<b>0.000</b>	0.089	0.932	0.985	1.000	0.967	<b>0.028</b>	1.000												
HID1	1.000	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.033</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.102	<b>0.000</b>	<b>0.000</b>											
HID4	0.913	0.864	<b>0.016</b>	0.447	1.000	0.990	0.948	0.258	0.975	1.000	0.381	<b>0.031</b>	0.095										
HID1.4	0.127	1.000	0.498	<b>0.013</b>	1.000	1.000	1.000	0.980	1.000	1.000	0.995	0.638	<b>0.001</b>	1.000									
LvD1	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.564	1.000	1.000	1.000	1.000	0.302	1.000	1.000	<b>0.000</b>	0.316	0.990								
LvD4	<b>0.004</b>	1.000	0.987	<b>0.000</b>	0.975	1.000	1.000	1.000	1.000	0.864	1.000	0.997	<b>0.000</b>	0.875	1.000	1.000							
LvD1.4	<b>0.000</b>	1.000	1.000	<b>0.000</b>	0.459	1.000	1.000	1.000	1.000	0.224	1.000	1.000	<b>0.000</b>	0.236	0.975	1.000	1.000						
PID1	<b>0.038</b>	1.000	0.789	<b>0.003</b>	1.000	1.000	1.000	0.999	1.000	0.995	1.000	0.886	<b>0.000</b>	0.996	1.000	1.000	1.000	0.999					
PID4	0.178	1.000	0.401	<b>0.020</b>	1.000	1.000	1.000	0.959	1.000	1.000	0.987	0.537	<b>0.001</b>	1.000	1.000	0.976	1.000	0.950	1.000				
PID1.4	0.389	0.999	0.185	0.067	1.000	1.000	1.000	0.816	1.000	1.000	0.909	0.281	<b>0.006</b>	1.000	1.000	0.867	0.999	0.792	1.000	1.000			
RaD1	1.000	0.156	<b>0.000</b>	0.989	1.000	0.455	0.267	<b>0.009</b>	0.353	1.000	<b>0.018</b>	<b>0.000</b>	0.760	1.000	0.829	<b>0.013</b>	0.165	<b>0.008</b>	0.551	0.893	0.983		
RaD4	0.971	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.004</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.015</b>	<b>0.000</b>	<b>0.000</b>	1.000	<b>0.014</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.001</b>	0.327	
RaD1.4	1.000	0.149	<b>0.000</b>	0.990	1.000	0.442	0.258	<b>0.008</b>	0.342	1.000	<b>0.017</b>	<b>0.000</b>	0.771	1.000	0.819	<b>0.012</b>	0.158	<b>0.007</b>	0.537	0.886	0.981	1.000	0.338



### 3.5.5 Community composition and spatial scale

#### 3.5.5.1 Community composition differs with depth

NMDS plots were generated from the AMF community composition and relative abundance data for each host plant. Grey envelopes were added to the NMDS plots to delineate the 2-dimensional space occupied by the AMF communities associated with each host plant. The relative locations of these envelopes and overlap between them were used as an indicator of the relative similarity of the AMF communities associated with each host plant species. Comparisons between the eight host plants amount to twenty-eight possible pairwise combinations.

#### 3.5.5.2 Both depths combined

There is considerable compositional and structural overlap between the AMF communities hosted by the eight plant species when data is pooled from both depths. Twenty-three of a possible twenty-eight pairwise comparisons between host plant species exhibited overlap (Figs. 3.19 & 3.20). When both depths are considered together, the *H. lanatus* communities only display overlap with those from *R. acetosa*, *A. odoratum* and *F. rubra*. There was no overlap between *P. lanceolata* and *F. rubra*. All other pairwise comparisons showed overlap.

#### 3.5.5.3 Depth 1

At depth 1, nineteen of a possible twenty-eight pairwise comparisons between host plant species exhibited overlap (Figs. 3.21 & 3.22); fewer than when data from both depths combined is considered. The AMF communities associated with *H. lanatus* appear to be distinct from those of any of the other host plants. No overlap existed

between *H. lanatus* communities and any of the other host plants. Additionally, there was no overlap between *A. odoratum* and either *L. vulgare* or *P. lanceolata*.

#### 3.5.5.4 Depth 4

At depth 4, only eleven of twenty-eight possible pairwise comparisons between host plant species exhibited overlap (Figs. 3.23 & 3.24). *C. jacea* communities did not overlap with those of *R. acetosa*, *L. vulgare*, *H. lanatus* or *F. rubra*. *R. acetosa* did not overlap with any of the other host plant species. *P. lanceolata* only overlapped with *C. jacea* and *A. capillaris*. *A. odoratum* only overlapped with *C. jacea* and *A. capillaris*.

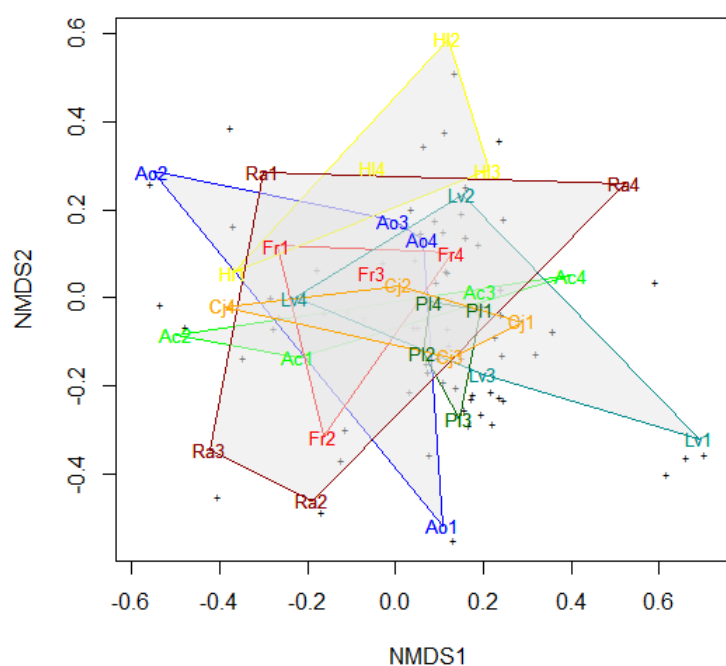


Figure 3.19. Nonmetric Multidimensional scaling plot for AMF communities in each plot of all host plant species, both depths combined. Codes and colours as follows: Ac (light green) = *Agrostis capillaris*, Ao (dark blue) = *Anthoxanthum odoratum*, Fr (red) = *Festuca rubra*, Hl (yellow) = *Holcus lanatus*, Cj (orange) = *Centaurea jacea*, Lv (light blue) = *Leucanthemum vulgare*, Pl (dark green) = *Plantago lanceolata* and Ra (brown) = *Rumex acetosa*.

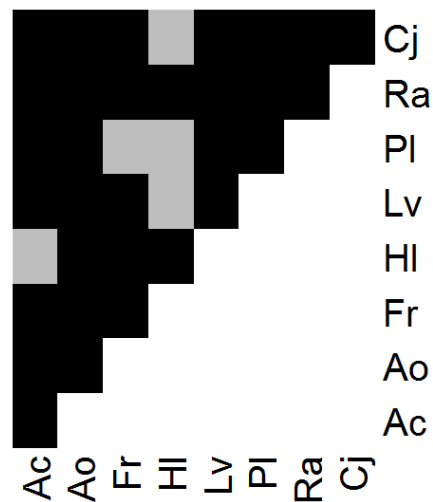


Figure 3.20. Summary presence-absence heatmap of overlap between 2-dimensional space on NMDS plot occupied by AMF communities associated with each host plant species (Fig. 3.19). Data from Depths 1 and 4 combined. Black cells indicate the presence of an overlap between host plants. Grey cells indicate no overlap. No data in lower-right half of the matrix.

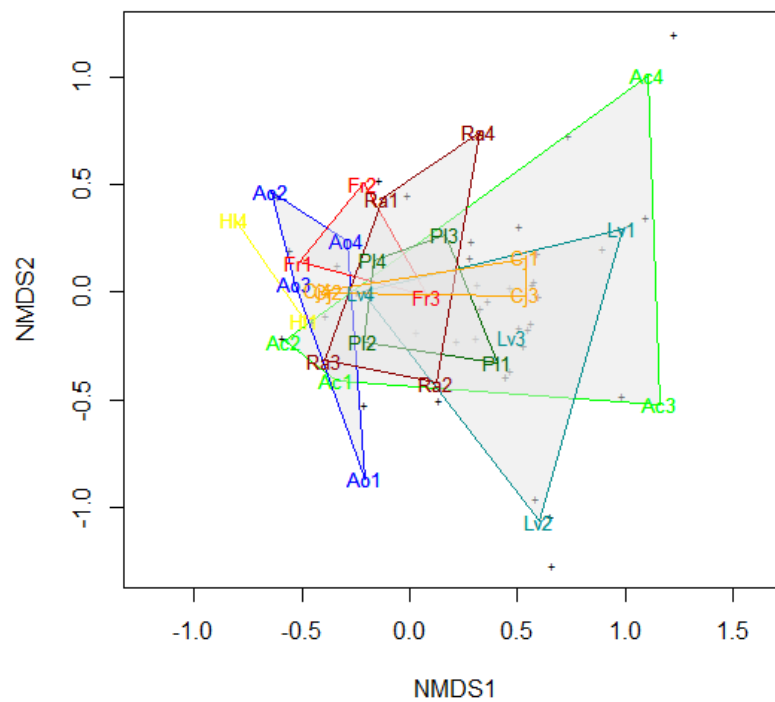


Figure 3.21. Nonmetric Multidimensional scaling plot for AMF communities in each plot of all host plant species, at depth 1. Codes and colours as follows: Ac (light green) = *Agrostis capillaris*, Ao (dark blue) = *Anthoxanthum odoratum*, Fr (red) = *Festuca rubra*, HI (yellow) = *Holcus lanatus*, Cj (orange) = *Centaurea jacea*, Lv (light blue) = *Leucanthemum vulgare*, PI (dark green) = *Plantago lanceolata* and Ra (brown) = *Rumex acetosa*.

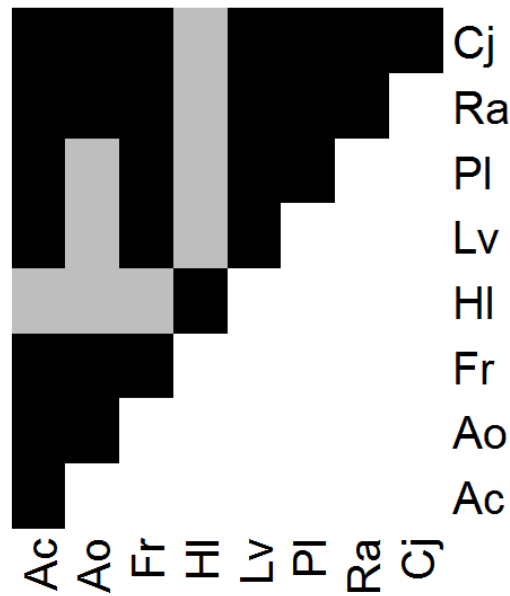


Figure 3.22. Summary presence-absence heatmap of overlap between 2-dimensional space on NMDS plot occupied by AMF communities associated with each host plant species (Fig. 3.21). Data from depth 1. Black cells indicate the presence of an overlap between host plants. Grey cells indicate no overlap. No data in lower-right half of the matrix.

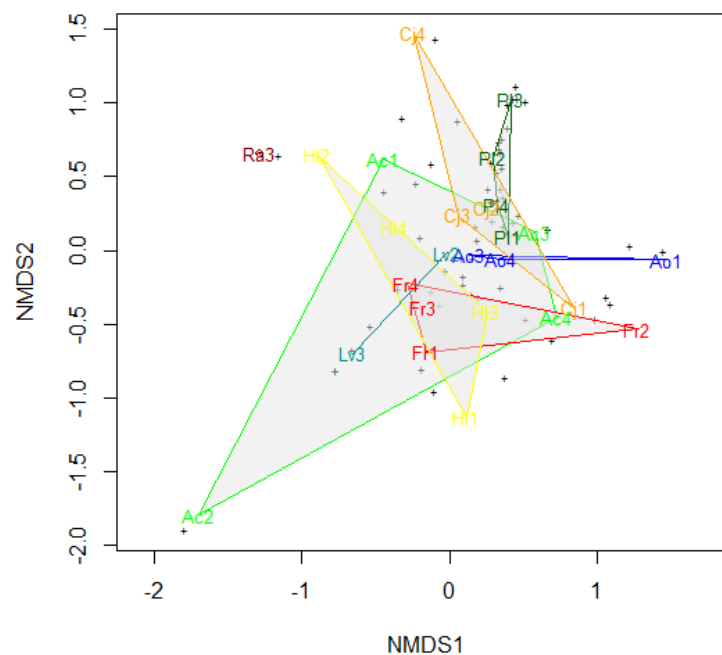


Figure 3.23. Nonmetric Multidimensional scaling plot for AMF communities in each plot of all host plant species, at depth 4. Codes and colours as follows: Ac (light green) = *Agrostis capillaris*, Ao (dark blue) = *Anthoxanthum odoratum*, Fr (red) = *Festuca rubra*, HI (yellow) = *Holcus lanatus*, Cj (orange) = *Centaurea jacea*, Lv (light blue) = *Leucanthemum vulgare*, PI (dark green) = *Plantago lanceolata* and Ra (brown) = *Rumex acetosa*.

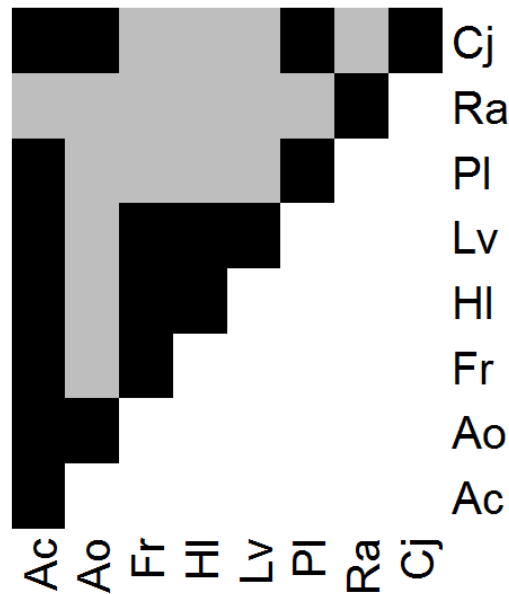


Figure 3.24. Summary presence-absence heatmap of overlap between 2-dimensional space on NMDS plot occupied by AMF communities associated with each host plant species (Fig 3.23). Data from depth 4. Black cells indicate the presence of an overlap between host plants. Grey cells indicate no overlap. No data in lower-right half of the matrix.

### 3.5.6 Nestedness of network depends on spatial scale

Many of the statistics describing AMF metacommunity bipartite networks depend on the scale at which the community is sampled. Each depth analysed alone yield different results from each other and from both depths analysed together (Tables 3.8 - 3.10 and Figs. 3.29 - 3.30). Using the qualitative NODF index, at Depth 1, the AMF communities associated with three host plant species were significantly nested (*P. lanceolata*, *R. acetosa* and *C. jacea*) (Table 3.9). At Depth 4, Four communities were significantly nested (*A. capillaris*, *F. rubra*, *P. lanceolata* and *C. jacea*) (Table 3.10). When data from depths 1 and 4 were combined, the AMF communities associated with five host plant species displayed significant nestedness (*A. capillaris*, *F. rubra*, *L. vulgare*, *P. lanceolata* and *C. jacea*) (Table 3.8). When the quantitative WNODF index was used on the same communities, all were found to be significantly less

nested than the simulated communities at all depths, apart from one, *H. lanatus*, which was significantly nested at depth 1 (Table 3.9).

At depth 1, the NODF metric was greater for columns than for rows in four plant species: *A. capillaris*, *A. odoratum*, *L. vulgare* and *R. acetosa*. For the other four species at this depth (*F. rubra*, *H. lanatus*, *P. lanceolata* and *C. jacea*), the reverse was true. At depth 4, the qualitative differences between row and column NODF was only conserved in three species. Column NODF was greater than row NODF in *A. capillaris*, *F. rubra* and *H. lanatus*, whereas row NODF was greater in *A. odoratum*, *L. vulgare*, *P. lanceolata* and *C. jacea*. The network for *R. acetosa* was too small at depth 4 to calculate nestedness. When the data from depths 1 and 4 were combined, the column NODF was greater than the row NODF in all plant species. The WNODF metric for columns was greater than the row WNODF in all plant species apart from *H. lanatus* at depth 1. At depth 4, column WNODF is greater than row WNODF in *A. capillaris*, *A. odoratum* and *P. lanceolata*, and row WNODF is greater than column WNODF in *F. rubra*, *H. lanatus*, *L. vulgare* and *C. jacea*. When both depths are combined, column WNODF is greater than row WNODF in all species apart from *H. lanatus*, for which the reverse is the case (Tables 3.8 - 3.10).

#### 3.5.6.1 Number of compartments depends on scale

*A. capillaris* communities exhibit three compartments in the network at depth 1 (Table 3.9), indicating three geographically separate AMF communities within the metacommunity. At depth 4 (Table 3.10) and when depths 1 and 4 are combined (Table 3.8), there are only two compartments in the *A. capillaris* network. Similarly, there are two compartments in the network of *A. odoratum* communities at depth 1 (Table 3.9) and when both depths are combined (Table 3.8), but only one at depth 4

(Table 3.10). *R. acetosa* communities are arranged into only one compartment at depth 1 (Table 3.9) and depth 4 (Table 3.10), but when both depths are combined (Table 3.8), they exhibit two compartments.

Table 3.8. Network properties for each host plant species using both depths combined. NODF = Nestedness metric based on Overlap and Decreasing Fill, C = column NODF, R = Row NODF, WNODF = Weighted Nestedness metric based on Overlap and Decreasing Fill. Significance (sig) for both nestedness metrics denoted by < (observed metric significantly smaller than simulated metacommunities), > (observed metric significantly greater than simulated metacommunities) and ns (observed metric not significantly different from simulated metacommunities).

Network property	<i>A. capillaris</i>	<i>A. odoratum</i>	<i>F. rubra</i>	<i>H. lanatus</i>	<i>L. vulgare</i>	<i>P. lanceolata</i>	<i>R. acetosa</i>	<i>C. jacea</i>
No. of compartments	2	2	1	1	1	1	2	1
compartment diversity	1.22	1.77	N/A	N/A	N/A	N/A	1.59	N/A
NODF	23.41	20.24	37.55	22.41	24.31	38.57	23.21	36.18
NODF sig (NODF, C, R)	>>>	ns ns ns	>>>	ns ns >	> ns >	>>>	ns ns ns	>>>
NODF by column	23.58	20.53	37.78	22.79	24.39	38.62	26.36	36.23
NODF by row	20.18	17.62	31.59	18.89	19.83	35.73	11.67	34.31
WNODF	16.44	12.44	21.54	11.81	13.98	22.60	9.64	16.74
WNODF sig	<	<	<	<	<	<	<	<
WNODF by column	16.57	12.63	21.91	11.40	14.02	22.71	10.91	16.74
WNODF by row	13.93	10.71	12.37	15.56	11.47	16.59	5.00	16.55
No. of AMF species	33	20	33	17	41	58	11	48
Difference NODF - WNODF	6.97	7.80	16.01	10.60	10.33	15.96	13.57	19.45
Matrix fill	0.16	0.24	0.27	0.24	0.25	0.27	0.24	0.23



Table 3.9. Network properties for each host plant species at Depth 1. NODF = Nestedness metric based on Overlap and Decreasing Fill, C = column NODF, R = Row NODF, WNODF = Weighted Nestedness metric based on Overlap and Decreasing Fill. Significance (sig) for both nestedness metrics denoted by < (observed metric significantly smaller than simulated metacommunities), > (observed metric significantly greater than simulated metacommunities) and ns (observed metric not significantly different from simulated metacommunities).

Network property	<i>A. capillaris</i>	<i>A. odoratum</i>	<i>F. rubra</i>	<i>H. lanatus</i>	<i>L. vulgare</i>	<i>P. lanceolata</i>	<i>R. acetosa</i>	<i>C. jacea</i>
No. of compartments	3	2	1	1	1	1	1	1
compartment diversity	2.61	1.70	N/A	N/A	N/A	N/A	N/A	N/A
NODF	13.24	34.38	44.37	57.14	16.97	31.39	31.86	39.74
NODF sig (NODF, C, R)	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	> > >	> > ns	> > >
NODF by column	14.29	40.00	43.64	50.00	17.01	31.05	32.22	39.54
NODF by row	8.33	25.00	57.78	100.00	13.61	42.22	29.17	50.40
WNODF	13.24	9.38	12.07	57.14	9.17	19.04	13.24	21.34
WNODF sig	<	<	<	>	<	<	<	<
WNODF by column	14.29	10.00	12.73	50	9.20	19.21	13.33	21.49
WNODF by row	8.33	8.33	0.00	100	7.50	13.52	12.50	13.49
No. of AMF species	8	5	11	4	30	20	10	26
Difference NODF - WNODF	0.00	25.00	32.30	0	7.79	12.36	18.63	18.39
Matrix fill	0.28	0.40	0.48	0.63	0.28	0.36	0.35	0.36

Table 3.10. Network properties for each host plant species at Depth 4. NODF = Nestedness metric based on Overlap and Decreasing Fill, C = column NODF, R = Row NODF, WNODF = Weighted Nestedness metric based on Overlap and Decreasing Fill. Significance (sig) for both nestedness metrics denoted by < (observed metric significantly smaller than simulated metacommunities), > (observed metric significantly greater than simulated metacommunities) and ns (observed metric not significantly different from simulated metacommunities).

Network property	<i>A. capillaris</i>	<i>A. odoratum</i>	<i>F. rubra</i>	<i>H. lanatus</i>	<i>L. vulgare</i>	<i>P. lanceolata</i>	<i>R. acetosa</i>	<i>C. jacea</i>
No. of compartments	2	1	1	1	1	1	N/A	1
compartment diversity	1.26	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NODF	27.77	35.93	39.93	28.87	30.04	54.73	N/A	53.14
NODF sig (NODF, C, R)	> > ns	ns ns ns	> > ns	ns ns ns	ns ns ns	> > >	N/A	> > >
NODF by column	27.78	35.83	40.00	29.12	30.04	54.70	N/A	53.13
NODF by row	27.50	40.00	34.87	25.00	30.77	61.12	N/A	54.41
WNODF	23.41	25.81	24.28	12.89	12.33	33.20	N/A	27.96
WNODF sig	<	<	<	<	<	<	N/A	<
WNODF by column	23.41	25.83	24.25	12.64	12.25	33.26	N/A	27.84
WNODF by row	23.33	25.00	26.09	16.67	30.77	21.41	N/A	38.56
No. of AMF species	28	16	30	14	23	48	N/A	33
Difference NODF - WNODF	4.36	10.12	15.65	15.98	17.72	21.54	N/A	25.18
Matrix fill	0.30	0.52	0.38	0.34	0.59	0.51	N/A	0.40



[illegible]

Figure 3.27. Maximally packed AMF OTU occurrence matrix for the *F. rubra* plots (Fr1-4) at both depths (D1+D4). Red text shows no. of 454 reads for each AMF OTU. Greyscale relates to AMF OTU abundance (in 454 reads)

[illegible]

Figure 3.28. Maximally packed AMF OTU presence-absence occurrence matrix for the *F. rubra* plots (Fr1-4) at both depths (D1+D4). Filled cells are presences and empty cells are absences.

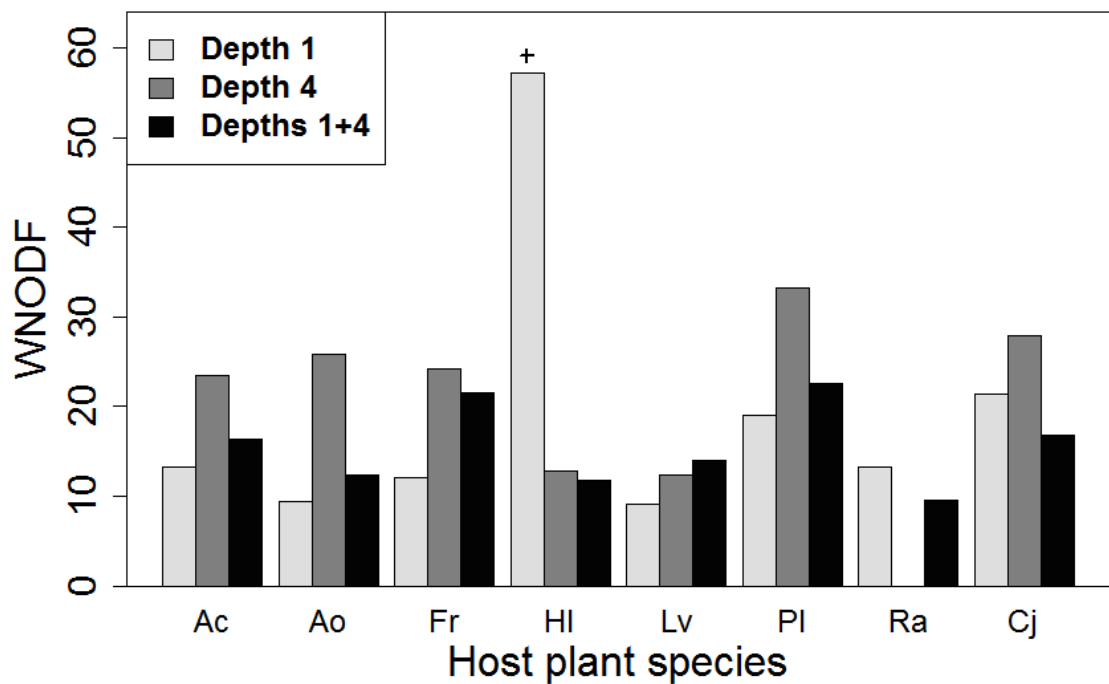


Figure 3.29. WNODF statistics for all host plant species at both depths separately and with both depths combined. Plus symbol indicates that the WNODF metric significantly greater than that of the simulated metacommunities. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

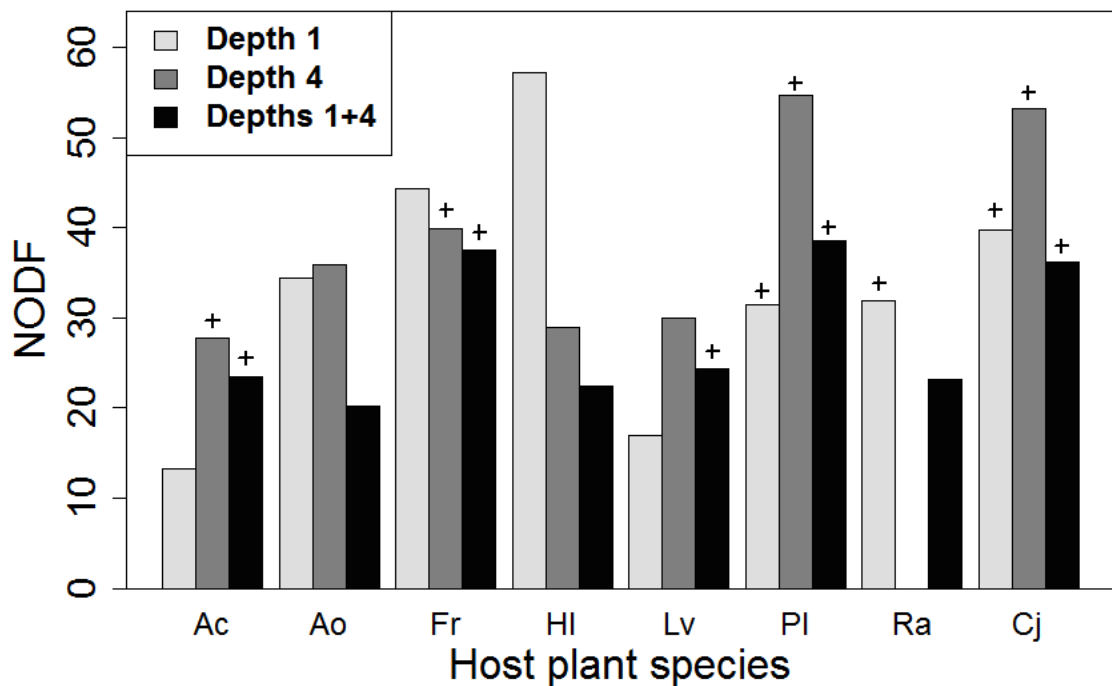


Figure 3.30. NODF statistics for all host plant species at both depths separately and with both depths combined. Plus symbol indicates that the WNODF metric significantly greater than that of the simulated metacommunities. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

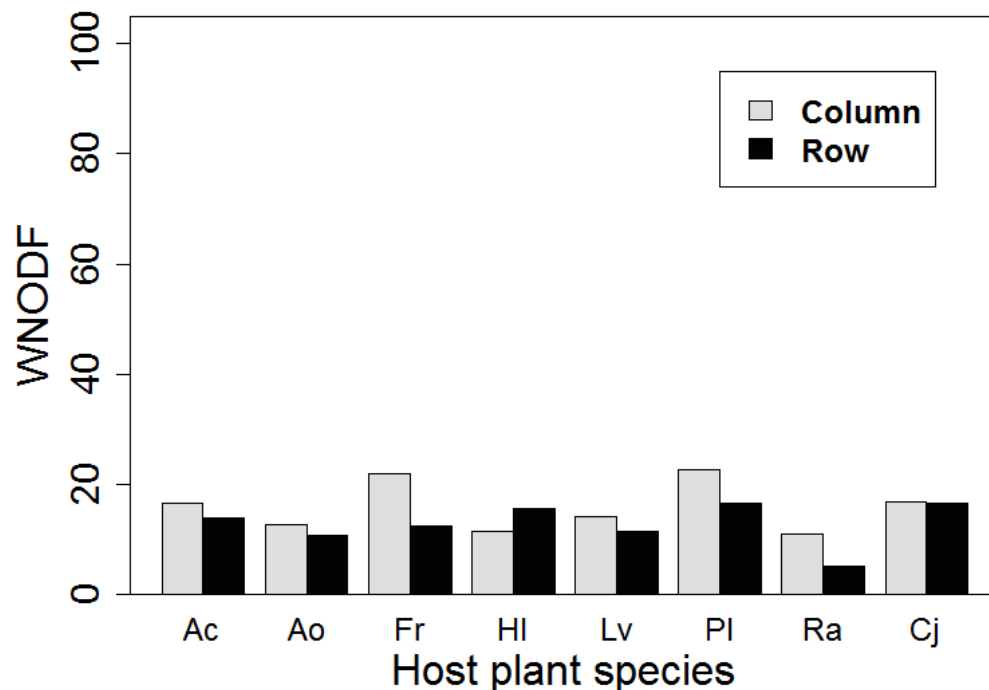


Figure 3.31. Column (grey) and row (black) WNODEF statistics for all host plant species in Depths 1 and 4 combined. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

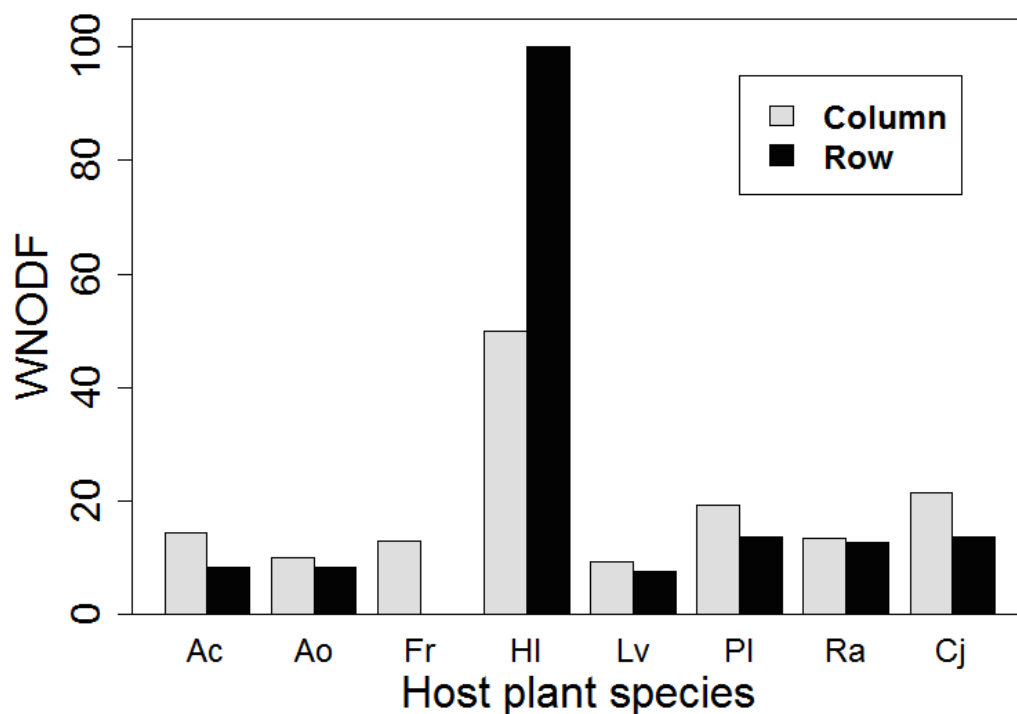


Figure 3.32. Column (grey) and row (black) WNODEF statistics for all host plant species at Depth 1. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

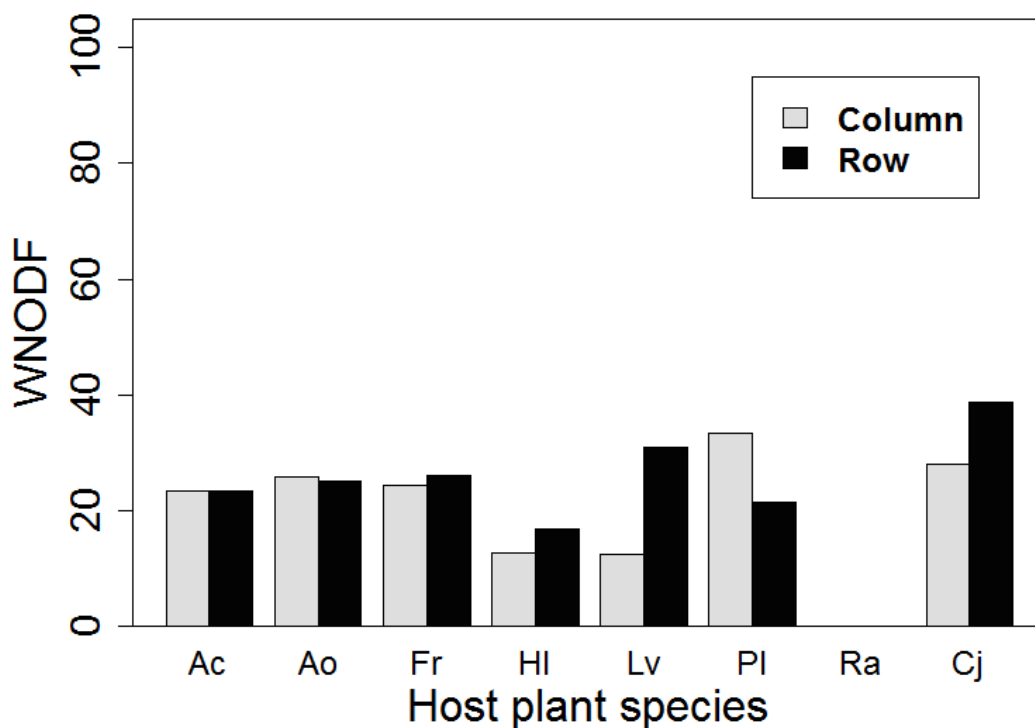


Figure 3.33. Column (grey) and row (black) WNODEF statistics for all host plant species at Depth 4. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

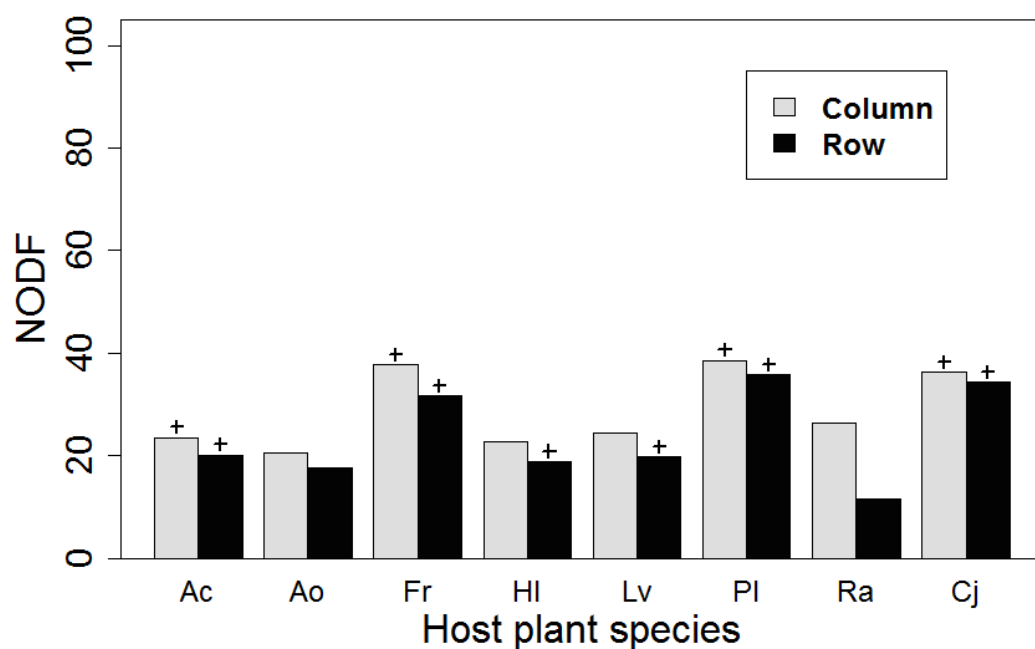


Figure 3.34. Column (grey) and row (black) NODF statistics for all host plant species in Depths 1 and 4 combined. Plus symbol indicates that the observed NODF metric is significantly greater than that of the simulated metacommunities. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

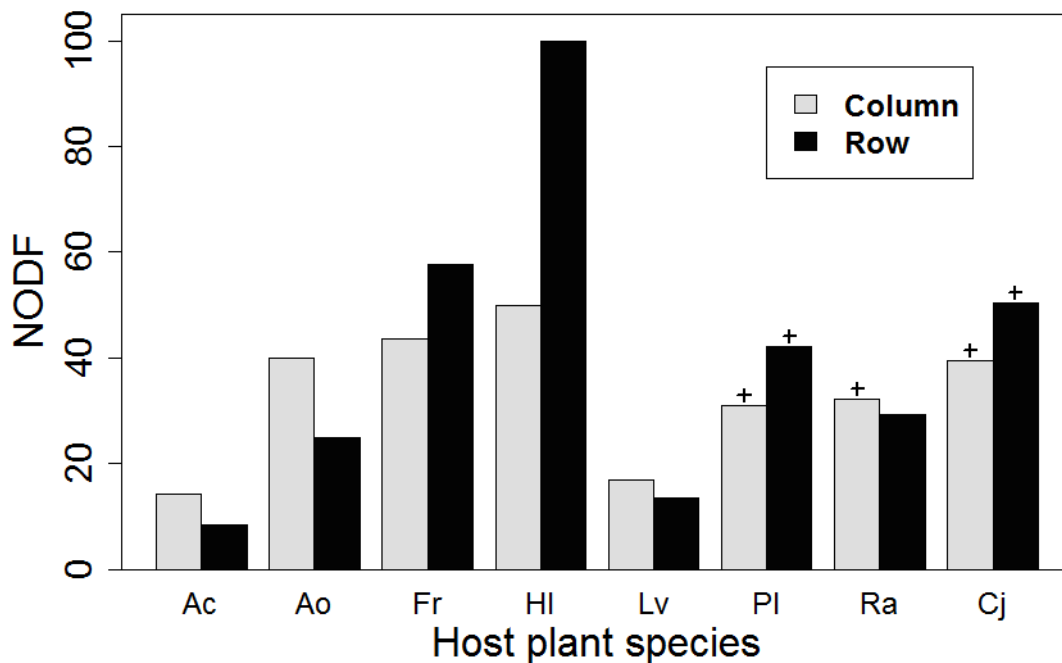


Figure 3.35. Column (grey) and row (black) NODF statistics for all host plant species at Depth 1. Plus symbol indicates that the observed NODF metric is significantly greater than that of the simulated metacommunities. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.

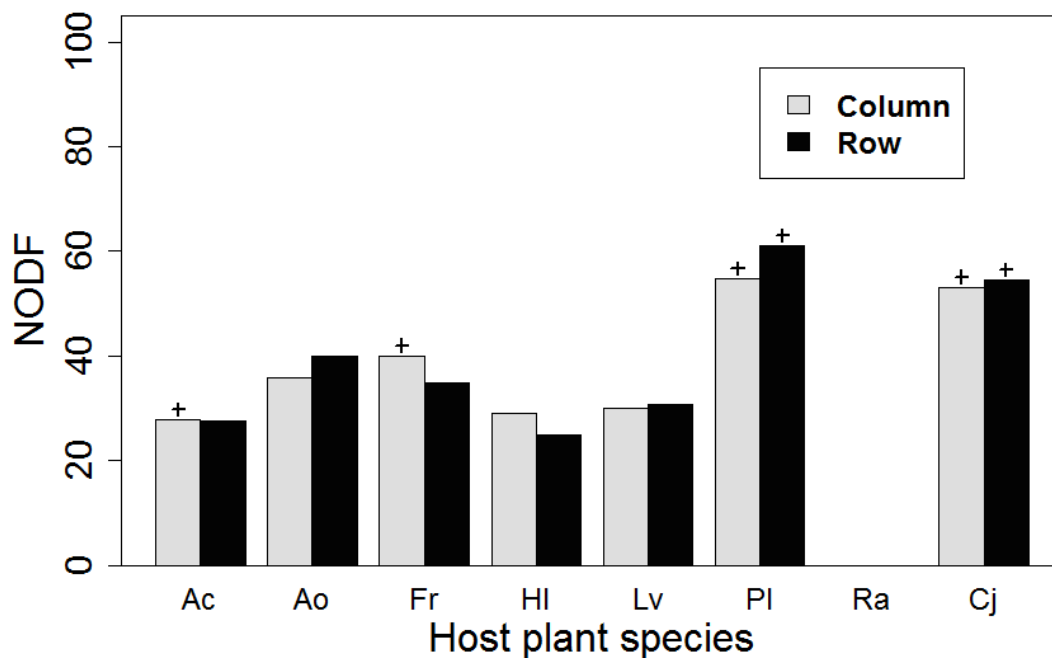


Figure 3.36. Column (grey) and row (black) NODF statistics for all host plant species at Depth 4. Plus symbol indicates that the observed NODF metric is significantly greater than that of the simulated metacommunities. Ac = *Agrostis capillaris*, Ao = *Anthoxanthum odoratum*, Fr = *Festuca rubra*, HI = *Holcus lanatus*, Lv = *Leucanthemum vulgare*, PI = *Plantago lanceolata* and Ra = *Rumex acetosa*, Cj = *Centaurea jacea*.



## 3.6 Discussion

### 3.6.1 Detection of nestedness depends on metric

Using the NODF metric, three, four and five of the eight host plant species hosted AM fungal metacommunities that were significantly nested at depth 1, 4 and in both depths combined respectively. The remainder of the metacommunities did not significantly differ from randomly generated matrices (Tables 3.8 - 3.10). Conversely, only one metacommunity sampled (*H. lanatus*, depth 1) yielded a significant degree of nestedness according to the WNODF metric. The matrices describing this metacommunity, however, were sufficiently small that the matrix produced using quantitative data and that produced with qualitative data did not differ in topography or structure. All other metacommunities tested apart from this one were significantly less nested than the simulated matrices. This result indicates that the nested patterns observed in the presence-absence matrices are not consistent with a pattern in which the populations of species in more species-poor communities are smaller than their conspecific populations in richer communities. This is the reason the order of rows (sites) differs between qualitative and quantitative matrices of the same metacommunity (Figs. 3.25 - 3.28). Additionally, this lack of WNODF nestedness in metacommunities recorded as nested by NODF indicates that the occupancy of a particular AM fungal OTU is not strongly positively correlated with its total abundance in the entire metacommunity, at least for the majority of OTUs detected. This is the reason the order of columns (AM fungal OTUs) differs between qualitative and quantitative matrices (Figs. 3.25 - 3.28). This pattern, in which the AM fungal communities are nested according to the qualitative metric, but not according to the quantitative metric, was observed in the only other study (to the authors'

knowledge) to compare results from the two metrics in AM fungal communities (Kawahara and Ezawa, 2013).

A positive feedback mechanism to which AM fungal communities are subject throughout the growing season is likely to contribute to these patterns. When the soil temperature is high enough at the start of the growing season, and fungal mycelia begin to extend throughout the soil, some mycelia encounter plant roots before the majority of the others. This is partly due to neutral processes (e.g. where the AM fungal propagules happen to be in relation to the potential plant hosts) and partly to niche-based processes (e.g. vital rates such as rates of mycelial growth or spore production) (Dumbrell *et al.*, 2010b). The AM fungal OTU to which the first mycelia to encounter a plant root belong receives the phytogenic carbon first, and with this resultant “head start”, is able to invest this carbon in further growth of its extra-radical mycelium (ERM). This increased growth rate in turn increases the likelihood that this OTU will encounter more plant roots and yet more phytogenic carbon, resulting in a positive feedback mechanism (Helgason and Fitter, 2009) that manifests as the uneven structure of AM fungal communities (Werner and Kiers, 2015).

The disparity between the quantitative and qualitative nestedness metrics could be indicative of a trade-off in AM fungal reproductive strategies. Those OTUs that invest in a greater rate of ERM growth may produce fewer, less dispersible or shorter-lived spores, whereas those that produce more spores and/or spores that are longer-lived or more easily dispersed may display lower rates of ERM growth. Such a trade-off would result in the decoupling of AM fungal OTU occupancy and abundance, as observed in the current study.

That the populations of AM fungal OTUs in less species-rich communities are generally larger than their conspecific populations in richer communities is evidence for a greater degree of unevenness in the less species-rich communities. It might be the case that these, less species-rich, communities are less rich because of the success of the most abundant OTUs. Werner and Kiers (2015) observed that, regardless of the identity of the first OTU to colonise a plant, the presence of an OTU within a plant root significantly decreased the abundance of any subsequent colonisers. The time lag between the first and second colonisations was also negatively correlated with the abundance of subsequent colonisers. Given that AM fungi can abort arbuscules in roots that do not provide sufficient carbon for uptake, in order to redirect their growth other plant roots (Javot *et al.*, 2007), the success of the first coloniser and time lag between first and subsequent colonisers is likely to contribute to the observed AM fungal community structure.

In addition to the greater ecological insight resulting from using both WNODF and NODF metrics in calculating nestedness, it is clear from these results that the matrix sorting parameters associated with NODF are put to their best use in detecting species with a greater occupancy (and thus those potentially with greater rates of dispersal), and that these may not be the species with a greater abundance. Studies which only use NODF should make this limitation explicit.

### *3.6.2 Niche-based processes affect the dominance structure of AM fungi more than neutral processes*

There was no difference in the rank order of AM fungal OTUs between 72% of the 276 pairwise comparisons tested (Tables 3.5 & 3.7). That the rank order of OTUs is conserved in the majority of metacommunities tested may indicate that niche-based

processes, such as inherent differences in certain vital rates between the AM fungal OTUs, afford the most common OTUs advantages which allow them to become more common than the rest. Nevertheless, 28% of pairwise comparisons were significantly different in rank order, indicating a role for stochastic processes. This result potentially adds evidence to the hypothesis that phenotypic differences among AM fungi are due to trade-offs in strategies; those AM fungal OTUs that germinate sooner or extend their ERM faster are more likely to gain the advantage from being the first to colonise a plant root than those that invest in, for example, longer-living spores at the expense of ERM extension rate. An alternative explanation is that those OTUs that have, through stochastic processes, gained an advantage in previous seasons by encountering plant roots first, have as a result gained a greater “propagule bank” which confers upon them an advantage in subsequent growing seasons. Dumbrell *et al* (2010a) observed an idiosyncratic pattern in AM fungal communities, in which a different OTU was most abundant in each community and thus concluded that stochastic processes largely determined the identity of the most abundant OTU. In the current study, a similar idiosyncratic pattern was not observed, with the same few AM fungal OTUs detected as the most abundant OTU in plots across the entire study area of >500m<sup>2</sup> (Figs 3.6 - 3.7 and Table 3.1). Therefore, it is more likely that the structure of AM fungal communities in the current study is determined to a greater degree by niche-based processes than neutral processes. Contrary to expectation, the spatial scale of sampling did not affect the proportion of pairwise comparisons that were significantly different in terms of rank order of AM fungal OTUs. At all scales in this study, therefore, niche-based processes appear to dominate in the structuring of AM fungal communities.

### *3.6.3 Spatial scale, depth of sampling and nestedness metric all affect the differences observed between row and column nestedness*

While column nestedness ( $WNODF_{(col)}$ ) was greater than row nestedness ( $WNODF_{(row)}$ ) in seven of the eight host plant species at depth one (Fig. 3.32), at depth four column nestedness was greater than row nestedness in only three of the seven host plants for which matrices were generated (Fig. 3.33). When both depths were combined, the same seven host plant species displayed greater column nestedness than row nestedness (Fig. 3.31). This suggests that when considering depths 1 and 4, opposite conclusions can be reached about whether differences between the AM fungal species in suitability to their habitat or differences between sites in their “quality” have a greater influence on the observed patterns in the community. When the NODF nestedness metric was used, column nestedness ( $NODF_{(col)}$ ) was greater than row nestedness ( $NODF_{(row)}$ ) in four of the eight host plant species at depth 1 (Fig. 3.35). At depth four, only three of the seven host plant species for which matrices were generated displayed greater column nestedness than row nestedness (Fig. 3.36). Greater column nestedness than row nestedness was observed in all eight of the host plant species when depths 1 and 4 were combined (Fig. 3.34). In contrast to the results for quantitative data, depths 1 and 4 were similar in the ratio of those communities with greater column nestedness to those with greater row nestedness. Depths 1 and 4 combined using qualitative data (NODF) yield the same result as the equivalent situation using quantitative data (WNODF), namely that the majority of plant species in this study host AM fungal communities whose structures are influenced to a greater degree by differences between the AM fungal OTUs, as opposed to differences between the sites. This conclusion is supported by the disparity between the quantitative and qualitative

nestedness metrics and by the relative similarity in rank order of AM fungal OTUs between communities. Given the relative homogeneity of the study plots, and the total diversity of the AM fungal community sampled, this finding is in accordance with expectations.

#### *3.6.4 The spatial scale and depth of sampling affects both qualitative and quantitative network descriptors*

Not only do the NODF and WNODF metrics produce scale-dependent results, but a number of other qualitative and quantitative network descriptors seem to be dependent on scale and depth of sampling as well. The number of compartments in a network, and as such, the compartment diversity, both qualitative descriptors, are highly depth and scale-dependent (Tables 3.8 - 3.10). In the current study the presence of more than one compartment in a network indicates endemism in certain AM fungal species. This could be a result of dispersal limitation in certain species, or spatial scales of sampling insufficient to detect the presence of these species elsewhere. In studies using interaction networks, as opposed to the incidence networks used in the current study, the presence of more than one compartment could indicate host plant preference or specificity in AM fungi or, again, insufficiently large spatial scales of sampling. When using either interaction or incidence networks, therefore, conclusions about certain aspects of the community ecology of AM fungi must necessarily be drawn from methodologies involving a range of spatial scales.

### **2.6.5 Experimental limitations and further work**

Determining how the patterns observed in the AM fungal communities profiled in the current study vary throughout time would provide further insight into the relative roles

of niche-based and neutral processes in the structuring of AM fungal communities. A multi-factorial experimental design, which includes a range of temporal and spatial scales, could potentially be the most informative way to investigate network properties of the AM fungal-plant mutualism. Additionally, the combined methods of nestedness analysis and dominance structure analysis applied in the current study could be useful when interaction networks, in which rows are host plant species and columns are AM fungal OTUs, are used in place of occurrence networks. Such an approach could be valuable in the investigation of host plant preference among AM fungi.

#### **2.6.6. Conclusions**

AM fungal communities are nested according to NODF, a qualitative nestedness metric, but not according to WNODF, its quantitative counterpart. This pattern is indicative both of greater unevenness in more species-poor communities and an absence of a correlation between AM fungal occupancy and abundance in the metacommunity. These are likely the result of (1) a positive feedback mechanism afforded to the earlier root colonisers and (2) a trade-off in dispersal ability, respectively. Niche-based processes affect the dominance structure of AM fungi to a greater degree than neutral processes, resulting in the majority of pairwise comparisons between rank order of AM fungal OTUs being statistically similar. Differences between AM fungal OTUs in vital rates and suitability to the habitat have a greater effect on community structure than differences between the sites when data from both depths was combined. However, the relative influence of site quality and AM fungal phenotype on community structure differed when data from each depth was considered alone. Additionally, the nestedness metric used influenced the

relative effect of these factors. The detection of local endemism among AM fungal OTUs was highly dependent on sampling depth and spatial scale.



## Chapter 4: Spatial patterning of the soil environment and its effect on natural AM fungal communities

### 4.1 Summary

- The compositional divergence of AM fungal communities in different host plant species as spatial scale increases could be a result of interspecific differences between host plants in the degree of environmental heterogeneity in their habitat.
- Soil environmental parameters that are known to affect the diversity, structure and composition of AM fungal communities were measured at three spatial scales ranging from 5 cm to >50 metres in undisturbed grassland habitat to determine the scale dependence of heterogeneity in the AM fungal microhabitat.
- There was considerable variation in all of the properties measured. Within the study site: Root biomass per soil core ranged varied from 0.01g to 2.07g, total soil organic carbon varied from 3.4% to 13.8%, dry bulk density varied from 0.31 g/cm<sup>3</sup> to 1.42 g/cm<sup>3</sup> and soil pH varied from 4.44 to 6.88, and by as much as 1.83 pH units among samples 20 cm apart.
- The soil environment surrounding *Leucanthemum vulgare* and *Festuca rubra* differed significantly in TOC, bulk density, pH and root biomass and the root biomass of *Festuca* plants was greater than that of *Leucanthemum*. This variation could explain host plant effects on AM fungal communities.
- There was only a difference in the degree of environmental heterogeneity in the soil samples surrounding each host plant species at large, but not at small, spatial scales.

- The detection of an interspecific difference in total organic carbon (TOC) content of soil samples was dependent on spatial scale, and thus might represent a causal mechanism for the scale dependence of host plant preference in AM fungi.

## 4.2 Introduction

### 4.2.1 Microbial Biogeography and environmental filtering

One fundamental aim in ecology is to understand the factors that determine the spatial distribution of organisms. Traditionally, however, biogeographical studies have been confined to macroorganisms, although relatively recent improvements in molecular techniques have facilitated the study of microbial diversity in unprecedented detail (Martiny *et al.*, 2006). Despite the influence microbial community diversity and composition is known to have over a wide range of ecosystem services, little was known about microbial biogeography until relatively recently (Naeem and Li, 1997, Balvanera *et al.*, 2006). It has long been assumed that microorganisms have such a high degree of vagility afforded to them by their small size and high abundance that no effect of dispersal limitation exists, and that their local diversity, driven by environmental parameters, varies little around the world (Bass-Becking, 1934). While evidence in support of this hypothesis has been found in certain microbial taxa (Finlay, 2002), in others, such as fungi (Green *et al.*, 2004) and bacteria (Van der Gast *et al.*, 2001), significant dispersal limitation has been observed at small spatial scales. This is suggestive of the action of mechanisms other than environmental filtering on community structure. Such distance decay of community similarity has been linked to environmental heterogeneity in bacteria (Horner-Devine *et al.*, 2004). The technological

improvements in sequencing technologies have revealed that microbial community composition, diversity and structure are influenced by a combination of historical contingency and contemporary environmental heterogeneity (Martiny *et al.*, 2006, Ge *et al.*, 2008). In this way, the factors affecting microorganism communities are similar to those affecting macroorganisms.

#### *4.2.2 Small-scale spatial patterns of arbuscular mycorrhizal fungi*

The AM fungi, similarly, have been observed to be affected by both past events such as dispersal limitation, extinction and speciation as well as local environmental conditions (van der Gast *et al.*, 2011, Hazard *et al.*, 2013). Even in habitats displaying a high degree of spatial heterogeneity, patterns suggestive of stochastic processes have been observed. Dumbrell *et al.* (2010b) recorded that AM fungal communities along a pH gradient fitted a zero sum multinomial species abundance distribution, the distribution predicted by neutral theory. The zero-sum assumption of neutral theory (Hubbell, 2001) states that there are never fewer individuals in a community than the environment permits, and that the environmental constraints on a community remain constant, resulting in a constant total number of competing individuals within a community (Etienne *et al.*, 2007). The zero-sum multinomial species abundance distribution, therefore, indicates that any difference in species composition between communities is a result of neutral, and not of niche-based processes (Dumbrell *et al.*, 2010b). The detection of such patterns may result from an inability to record a deterministic response due to insufficiently fine-scale methods used to measure the environmental variables within the habitat of the AM fungi. Investigation of the spatial patterning of the niche and the heterogeneity of environmental variables known to influence AM fungal communities at a range of spatial scales could reveal the spatial dependence of niche-based processes that

structure these communities. While distance decay in AM fungal community similarity has been observed at larger spatial scales (van der Gast *et al.*, 2011), evidence from many studies worldwide supports the hypothesis that environmental parameters are of greater importance at smaller spatial scales (Lekberg *et al.*, 2007, Dumbrell *et al.*, 2010b, Lumini *et al.*, 2010). Indeed, such is the importance of environmental heterogeneity in the structuring of AM fungal communities that a mosaic of soil types has been suggested as an important feature of the landscape in maintaining high  $\beta$ -diversity in AM fungal metacommunities (Lekberg *et al.*, 2007). It is clear that deterministic (niche-based) and stochastic (neutral) processes interact to determine diversity, composition and structure of AM fungal communities and that the relative influence of each is dependent on the habitat under study. So while, using Bass-Becking's phrase, the environment does seem to select, so too do the stochastic processes that caused the AM fungal propagules to be there in the first place. How the spatial scale of observation affects the perceived relative influence of these processes is less clear.

#### *4.2.3 Environmental filtering in AM fungal communities*

AM fungi inhabit both plant roots and the surrounding soil. The AM fungal extra-radical mycelium is involved in nutrient uptake from the soil, its branched structure increasing the surface area for nutrient exchange (Bago *et al.*, 2004). As such, the majority of AM fungal biomass exists in the soil environment (Smith and Read, 2008), and so a wide range of soil physical properties can influence AM fungal communities. Many of these properties are interdependent, rendering the study of their spatial patterns a complicated process. Nevertheless, investigating how these soil properties vary throughout space will provide important insight into the mechanisms structuring AM fungal communities.

#### 4.2.3.1 Soil pH

Soil pH has repeatedly been shown to be an important factor in structuring AM fungal communities. There is considerable difference among the AM fungi in the effect of soil pH on vital rates, such as root colonisation, mycelial growth and spore formation and germination (Porter *et al.*, 1987, van Aarle *et al.*, 2002). In an area where the soil pH is very heterogeneous, the diversity and composition of AM fungal communities have been shown to be affected most significantly by pH, followed by soil C/N ratio, host plant composition and soil phosphorus (Dumbrell *et al.*, 2010b). Indeed, Dumbrell *et al.* (2010b) observed that variance of pH positively correlated with AM fungal Beta diversity, suggesting that a greater heterogeneity in pH leads to greater diversity of the AM fungal metacommunity. Apart from the direct effects on AM fungal vital rates, the pH of the soil may affect AM fungi by mediating other factors, such as plant community composition (Lekberg *et al.*, 2011) and nutrient availability (Brady and Weil, 1996). The solubility of phosphate compounds is affected by soil pH (Black, 1943), and in turn there is a negative relationship between phosphorus availability and AM fungal root colonisation (Miller *et al.*, 1995).

#### 4.2.3.2 Organic matter content of soil

Read (1991) hypothesized that the organic matter content of soil would be a determinant of frequency of plants associating with AM fungi. He suggested that, while ectomycorrhizal and ericoid mycorrhizal fungi can break down labile organic nutrients and the more recalcitrant organic compounds respectively, AM fungi possess limited ability to degrade organic matter, and so AM plants should be more abundant in soil ecosystems with smaller pools of organic nutrients. Treseder and Cross (2006) found, in a meta-analysis of 151 geographical locations from 9 biomes,

that AM abundance, host plant availability and plant allocation to AM fungi (measured in percentage root length colonised) did not vary significantly with any of the following three measures of soil organic matter: Soil organic matter (SOM) content (amount of nutrients stored in organic form), input (the rate at which organic nutrients become available to AM fungi and their host plants) or residence time (a measure of recalcitrance of organic nutrients). Results of experimental studies are varied, however. Some suggest that hyphal growth rates of AM fungi in soils with a higher organic matter content are greater (St. John *et al.*, 1983, Joner and Jakobsen, 1995), and some suggest that hyphal growth rates could be suppressed in soils with a higher organic matter content (Ravnskov *et al.*, 1999). These differing conclusions are, perhaps, not surprising considering the factors that may confound the results of such studies. The organic matter content of soil can affect communities of other soil biota, such as saprophytic fungi like *Trichoderma*, which can enhance spore germination and hyphal growth in AM fungi (Calvet *et al.*, 1992). The type of organic matter added to soil in experimental studies can affect AM fungal communities by changing the particle size and grain size frequency distribution of the growing medium (Gaur and Adholeya, 2000). Colonisation of plant roots can be greater in a growing medium comprised of smaller particles, and both organic matter and nutrient availability are affected by soil texture (Gaur and Adholeya, 2000). As one of the most important factors in partitioning the niche space of AM fungi, it is likely that heterogeneity in the total organic carbon content (TOC) content of soil affects the structure and composition of AM fungal communities significantly. As such, any scale dependence in the heterogeneity of this soil property is likely to render conclusions drawn about AM fungal community structure dependent upon the spatial scale of sampling.

#### 4.2.3.3 Bulk density

Lekberg *et al.* (2007) reported a significant difference in AM fungal community composition between soils with different bulk densities. Gigasporaceae species dominated in soils with a lower bulk density (sandy soils) whereas Glomeraceae species predominated in soils of a greater bulk density (clay soils). The authors concluded that the possibility that parameters such as climate, host plant identity, management practices and season were contributory factors was minimal, due to the homogeneous nature of the study site. However, they conceded that, due to the interdependent nature of soil physical properties, deducing the causal factors behind the patterns observed is not a straightforward pursuit. Differences in vital rates between the AM fungal families, such as biomass allocation and growth rates of the extraradical hyphae have been detected (Dodd *et al.*, 2000, Hart and Reader, 2002) and are likely to contribute to the observed patterns.

#### 4.2.3.4 Host plant identity

Hazard *et al.* (2013) reported non-random AM fungal communities in the roots of two co-occurring plant species, *Lolium perenne* and *Trifolium repens*. These distinct AM fungal assemblages were consistent across all sites sampled, the distance between which varied from 7 to 392km. The plots from which the plants were collected in this study were 30 x 30 metres. Santos *et al.* (2006), whose experimental design incorporated plots which were much smaller and closer together (1.5 metre diameter plots on 4 x 40 m transects which were 6 metres apart) found no evidence for a host plant preference among AM fungi. Similarly, Öpik *et al.* (2003), in a reciprocal seedling establishment experiment using two co-occurring species of *Pulsatilla* (one rare, one common) and soil inocula from sites that supported both or only the

common species, failed to find a host plant preference. They did, however, report a site-dependent difference in AM fungal community composition. Sýkorová et al. (2007b) reported that host plant identity had a significant effect on AM fungal community composition in two co-occurring species of *Gentiana* at two sites 600 metres apart. They found no effect of site on AM fungal community composition. The sites in this study were 40 and 30 metres in diameter. It seems that host plant identity has an effect on the associated AM fungal community composition only at larger spatial scales. This could be because the preferred habitats of these host plants display different degrees of heterogeneity, which influence the composition of their associated fungal communities, only at certain scales.

#### *4.2.4 Spatial heterogeneity in the AM fungal microhabitat*

Given the obligate nature of the mutualism on the part of the fungus, environmental parameters that are known to affect plant distributions are likely to affect their associated AM fungal communities. The majority of AM fungal biomass is in the soil matrix, however, and as such, fungal communities are subject to the many soil environmental variables that influence their host plants. Jackson and Caldwell (1993) observed a high degree of heterogeneity in pH and soil organic matter at scales of less than 1 metre. The smallest spatial scale of this study was 12.5 cm, and given that the extraradical mycelium of some AM fungal species does not extend this far (Schubert *et al.*, 1987, Bago *et al.*, 2004), investigation of the heterogeneity and absolute values of soil environmental variables at smaller scales than this would be useful in addressing questions about the factors that influence AM fungal spatial patterns. Little research has been done on how variable the AM fungal niche space is at these very small spatial scales and how host plant identity affects the heterogeneity to which their associated AM fungal communities are subject. Such



research could provide insight into the spatial variation of the soil environmental variables which influence AM fungal communities the most, and thus potentially inform management practice to maximise diversity.

### **4.3 Aims**

In this study the following hypotheses are being tested:

1. Different host plant species provide their AM fungal communities with different microhabitats, due to interspecific differences in plant physical characteristics and preferences in soil environmental variables.
2. Differences between the host plant species in soil environmental parameters of their AM fungal habitat are only detectable at certain spatial scales.
3. Plant species differ in the degree of environmental heterogeneity to which their associated AM fungal communities are subject
4. Difference in environmental heterogeneity between plant species are dependent on spatial scale of sampling

Aims:

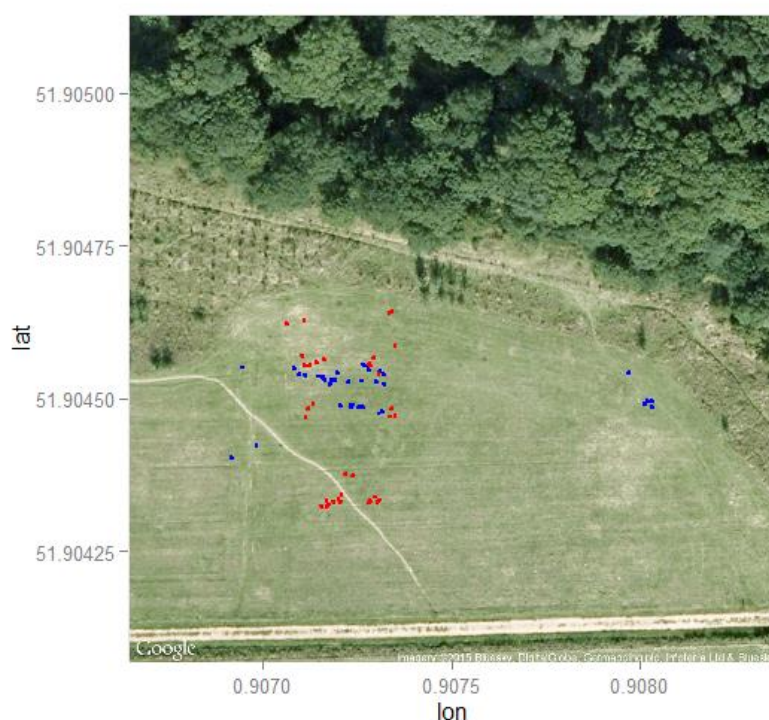
5. To quantify the degree of spatial heterogeneity within the AM fungal habitat
6. To determine whether the difference in spatial heterogeneity between host plant species could contribute to the detection of host plant preference

### **4.4 Materials and methods**

#### *4.4.1 Sample collection*

In order to investigate the scale dependency of heterogeneity within the habitat of AM fungi and its influence on AM fungal community structure, plant physical

properties and soil physicochemical characteristics were recorded from a grassland ecosystem. The grassland ecosystem was in an unmowed field (co-ordinates: 51.904357, 0.907676) on London clay, dominated by *Holcus lanatus*, *Festuca rubra* and *Pulicaria dysenterica*, within High Woods Country Park in Colchester, Essex. High Woods Country Park consists of 150 ha of mixed habitats, including woodland, marsh, grassland and scrubland. A preliminary search located all *Festuca rubra* and *Leucanthemum vulgare* plants with aboveground parts visible. These plants were numbered and their position recorded. Seventy plants (Thirty-five whole *Festuca rubra* individuals and thirty-five *Leucanthemum vulgare* individuals) were randomly selected and collected for analysis (Fig. 4.1). Eight soil cores from 0 - 8cm in depth and 3cm diameter were taken from around each plant. In order to incorporate a wide range of distances between which the heterogeneity of the environmental parameters could be analysed, two cores in each direction corresponding to North, East, South and West of each plant, at 5 cm and 10 cm from the plant were collected



(Fig. 4.2). This experimental design provides three basic spatial scales: 5cm distance between neighbouring pairs, 20cm distance between all eight cores surrounding each plant and up to ~50 metres distance between the most distant plants sampled.

Figure 4.1. Locations of plants collected at High woods country park, Colchester, Essex. Blue points are *Leucanthemum vulgare* and red points are *Festuca rubra* plants. lon = Longitude, lat = Latitude.

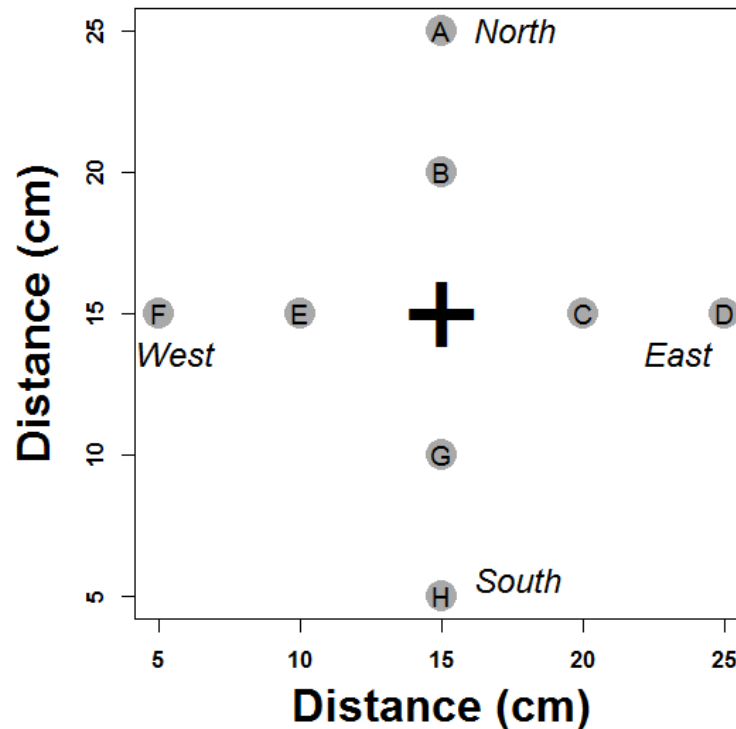


Figure 4.2. Core sampling pattern. Grey circles are core samples, labelled A-H. Black plus symbol is the location of the plant around which the cores were taken.

#### 4.4.2 Soil analysis

Rhizosphere soil was removed from the roots of the thirty-five *Leucanthemum* and thirty-five *Festuca* plants (Fig. 4.1). These soil samples, hereafter referred to as plant-soil samples, were dried to a constant weight in a forced air oven and stored for pH and TOC analysis. Soil samples taken from the soil cores surrounding each plant (Fig. 4.2), hereafter referred to as core-soil samples, were weighed to an accuracy of 0.01 g, dried to constant weight at 70°C in a forced air oven, weighed again to determine water content and bulk density, then sieved using a 2mm soil sieve. Root fragments recovered from the sieving of the core-soil samples were weighed to determine root biomass per core. After drying, the plant-soil and the core-soil samples were stored in airtight containers for no more than 6 weeks, until further analysis was carried out.

#### *4.4.2.1 Plant physiology*

The whole plant individuals were dried to a constant weight at 70°C in a forced air oven, separated into roots and shoots and the respective components weighed to an accuracy of 0.01 g to determine absolute biomass and root:shoot ratios.

#### *4.4.2.2 Total Organic Carbon (TOC)*

The ash-free dry weight method was used to determine the total organic carbon (TOC) content of soil in plant-soil and core-soil samples. For each soil sample, ~5g of sieved soil was weighed, and the organic content of the soil was burned off in a muffle furnace at 500°C for five hours. The ash-free soil was weighed again and the total organic carbon (TOC) content of the soil was determined.

#### *4.4.2.3 pH analysis*

3g of sieved soil and 15ml 0.01 Molar CaCl<sub>2</sub> (calcium chloride) homogenised for 30 seconds, left to settle for a minute, then pH was measured to two decimal places using a Jenway 3510 pH probe with calibration points at pH 4 and pH 7 (Bibby Scientific, Staffordshire, UK). Calcium chloride was used instead of water to provide greater precision in pH measurements, as it is less affected by electrolyte concentration in soil and therefore provides a more accurate approximation of the pH of the soil solution under field conditions (Minasny *et al.*, 2011).

#### *4.4.3 Molecular methods*

The washed, dried fine roots from each of the seventy whole plants were homogenised using stainless steel beads in microcentrifuge tubes on a TissueLyser II (Qiagen Ltd, W Sussex, UK). DNA was extracted from the High Woods plant roots

using MoBio PowerPlant DNA isolation kit following the manufacturer's instructions (Mo Bio Laboratories Inc., Carlsbad, CA, USA).

#### 4.4.3.1 Amplicon PCR and Clean-up

Illumina adapter overhang nucleotide sequences (Illumina, Madison, WI, USA) were added to the universal eukaryotic primer NS31 (Simon *et al.*, 1992) and the primer AM1 which amplifies the major Glomeromycotan families (Helgason *et al.*, 1998) (Table ). A 550 bp partial fragment of the AM fungal Small Subunit (SSU) ribosomal RNA gene was amplified using the modified primers.

Table 4.1. Primer sequences with Illumina adapter overhangs. Direction is 5' - 3'

Primer	Sequence
Forward (NS31)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGGAGGGCAAGTCTGGTGCC
Reverse (AM1)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTCCCGTAAGGCGCCGAA

The Amplicon PCR reactions were carried out in the presence of 0.2  $\mu$ M of each primer in the KAPA HiFi HotStart DNA Polymerase ReadyMix reaction buffer (2X: 0.3 mM of each dNTP, 2.5 mM  $MgCl_2$ ) and 2.5  $\mu$ l template DNA (5ng/ $\mu$ l) in 25  $\mu$ l reactions (PCR conditions: 95 °C for 3 min; 32 cycles at 98 °C for 0.5 min; 62 °C for 0.5 min and 72 °C for 1 min; and 72 °C for 10 min) on an Eppendorf Mastercycler® personal (Eppendorf Hamburg, Germany). To remove humic-acid-based PCR inhibitors, 0.05  $\mu$ L of T4 gene 32 protein (Roche Diagnostics Ltd,W. Sussex, UK) was added to all PCR reactions. Reactions resulting in a single PCR product were purified using Agencourt AMPure XP PCR Purification Kit (Beckman Coulter (UK) Ltd, High Wycombe, UK). Where PCR reactions resulted in different-sized amplicons when run on an agarose gel, the target amplicon band was isolated using a Qiaquick gel extraction kit following the manufacturer's instructions (Qiagen Ltd, W Sussex,

UK). For samples whose target amplicon failed to amplify sufficiently, the DNA template was reamplified with the higher annealing temperature of 64°C. The clean-up process for these samples was selected based on the criteria outlined above for the amplicon PCR. If the quantity of PCR product was still insufficient (based on the brightness of bands' fluorescence under UV light after running on an Agarose gel and stained with ethidium bromide, two PCR reactions of the same sample were pooled and cleaned using Qiaquick PCR purification kit (Qiagen Ltd, W Sussex, UK).

For the Index PCR, 5 µl of product from the amplicon PCR was used as template and reactions were carried out in the presence of 5 µl of each of the Nextera index primers with 25 µl KAPA HiFi HotStart DNA Polymerase ReadyMix reaction buffer (2X: 0.3 mM of each dNTP, 2.5 mM MgCl<sub>2</sub>) and 10 µl of water. Reactions were purified using the Agencourt AMPure XP PCR Purification Kit, then quantified using a Nanodrop 3300 (Thermo Scientific, Wilmington, DE, USA). Samples were diluted to the same concentration and pooled. The amplicon libraries were quality checked using a DNA 1000 kit on a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) before sequencing was performed on the Illumina MiSeq platform using a MiSeq reagent kit V3 (2 × 300bp) at TGAC (The Genome Analysis Centre, Norwich, UK).

#### *4.4.4 Data analysis*

##### *4.4.4.1 Spatial analysis*

To determine the co-ordinates of each soil core, the fossil package (Vavrek, 2011) in the R statistical language was used (R-Development-Core-Team, 2011). Geographical heatmaps were produced with the following packages: maps (Brownrigg, 2014b), maptools (Bivand, 2015b), RColorBrewer (Neuwirth, 2014), classInt (Bivand, 2015a) and mapdata (Brownrigg, 2014a). The spatial dependence

of the heterogeneity of each soil environmental parameter was assessed using the mark correlation function (Penttinen *et al.*, 1992) in the spatstat package (See Chapter 2 for further details). The small spatial scale results refer to values for the mean, range and variance between neighbouring soil cores (when only core-soil samples used: A and B, C and D, E and F, G and H and when core-soil and plant-soil samples used: P (plant-soil) and B, P and C, P and E and P and G, and all these comparisons when both core-soil and plant-soil samples were used, Fig. 4.2). The intermediate spatial scale results refer to the mean, range and variance of the 8 soil cores surrounding each plant individual (Fig. 4.2),

#### 4.4.4.2 Data transformation

The plant physical data (root, shoot and whole plant dry weight and root:shoot ratio) were not normally distributed in either host plant species according to a Shapiro-Wilk normality test. These data were log-transformed to achieve normality before being tested with analysis of variance.

## 4.5 Results

### 4.5.1 Physical properties of the host plants *Festuca rubra* and *Leucanthemum vulgare* differ

The root biomass of *F. rubra* plants was significantly greater than that of *L. vulgare* ( $F_{1, 68} = 7.455$ ,  $P = 0.0081$ ) (Fig. 4.3(a)). While the shoot biomass (Fig. 4.3(b)) and the biomass of the whole plant (Fig. 4.3(c)) did not differ between plant species ( $F_{1, 68} = 1.249$ ,  $P = 0.27$ ;  $F_{1, 68} = 0.631$ ,  $P = 0.43$  respectively), the root:shoot ratio was significantly greater in *F. rubra* than in *L. vulgare* plants ( $F_{1, 68} = 30.81$ ,  $P < 0.0001$ ) (Fig. 4.3(d)).

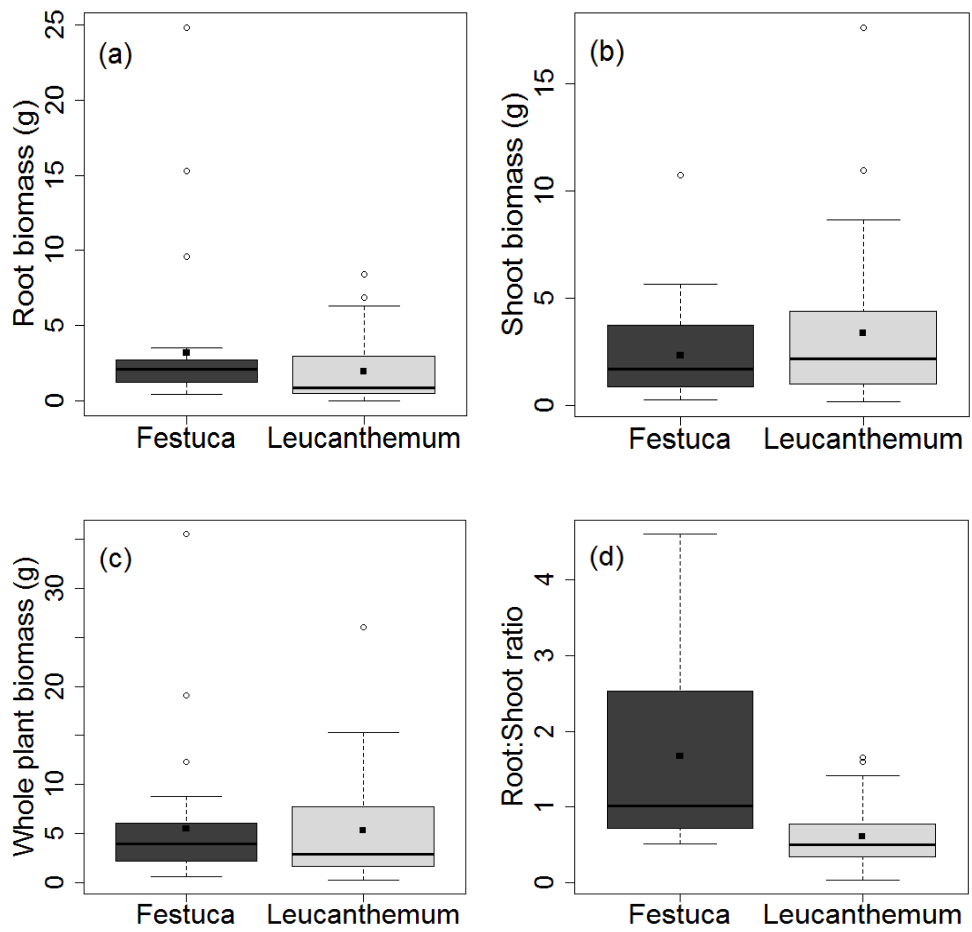


Figure 4.3. Plant physical characteristics of *Festuca rubra* (dark grey) and *Leucanthemum vulgare* (light grey). Black squares are mean values. (a) Root biomass, (b) Shoot biomass, (c) Biomass of the whole plant individual and (d) Root:shoot ratio.



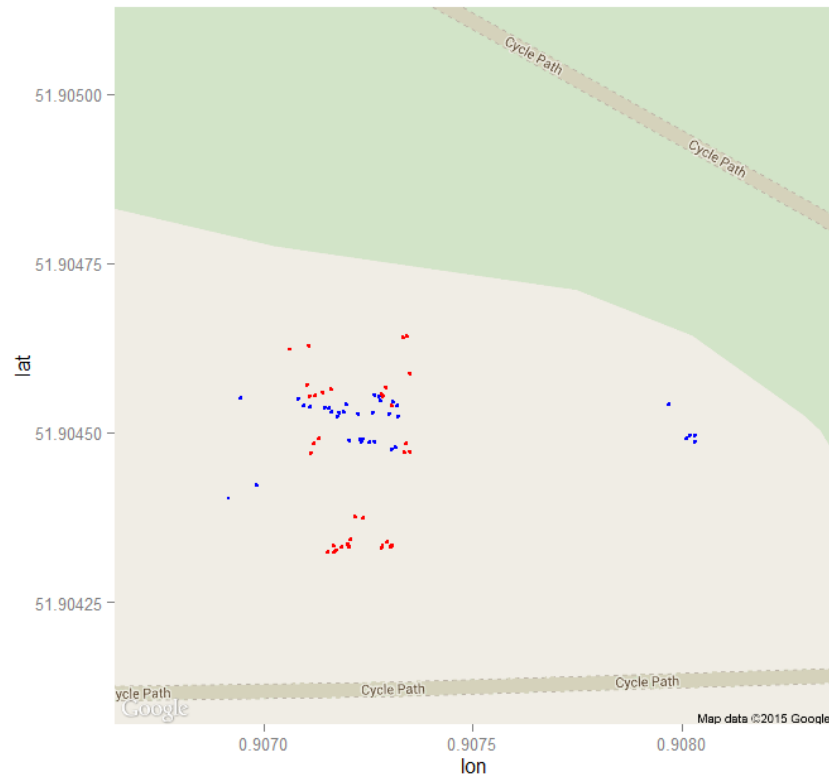


Figure 4.4. Locations of plants and surrounding soil cores collected at High woods country park, Colchester, Essex. (Red = *Festuca rubra*, Blue = *Leucanthemum vulgare*). lon = Longitude, lat = Latitude.

#### 4.5.2 Soil data

##### 4.5.2.1 Root biomass is greater and is more heterogeneous in the AM fungal habitat around *F. rubra*

Mean root biomass per core-soil sample was significantly greater in the *Festuca* soil cores than the *Leucanthemum* cores when data from all cores were analysed separately ( $F_{1,558} = 58.73$ ,  $P < 0.0001$ ), when the means of neighbouring pairs (A and B, C and D, E and F, G and H, see Fig. 4.2) were analysed ( $F_{1,278} = 41.3$ ,  $P < 0.0001$ ) (Fig. 4.5 (a)) and when the means of the eight cores surrounding each plant were analysed ( $F_{1,68} = 17.8$ ,  $P < 0.0001$ ) (Fig. 4.5 (b)). The range in root biomass between neighbouring pairs of cores was also significantly greater in *Festuca* than in *Leucanthemum* ( $F_{1,278} = 10.4$ ,  $P = 0.001$ ) (Fig. 4.5 (c)), as was the range between

the eight cores surrounding each plant ( $F_{1,68} = 9.87$ ,  $P = 0.002$ ) (Fig. 4.5 (d)). The variance of the root biomass between neighbouring pairs of cores was significantly greater in *Festuca* than in *Leucanthemum* ( $F_{1,278} = 6.05$ ,  $P = 0.015$ ) (Fig. 4.5 (e)), as was the variance for the eight cores surrounding each plant ( $F_{1,68} = 6.93$ ,  $P = 0.011$ ) (Fig. 4.5 (f)).

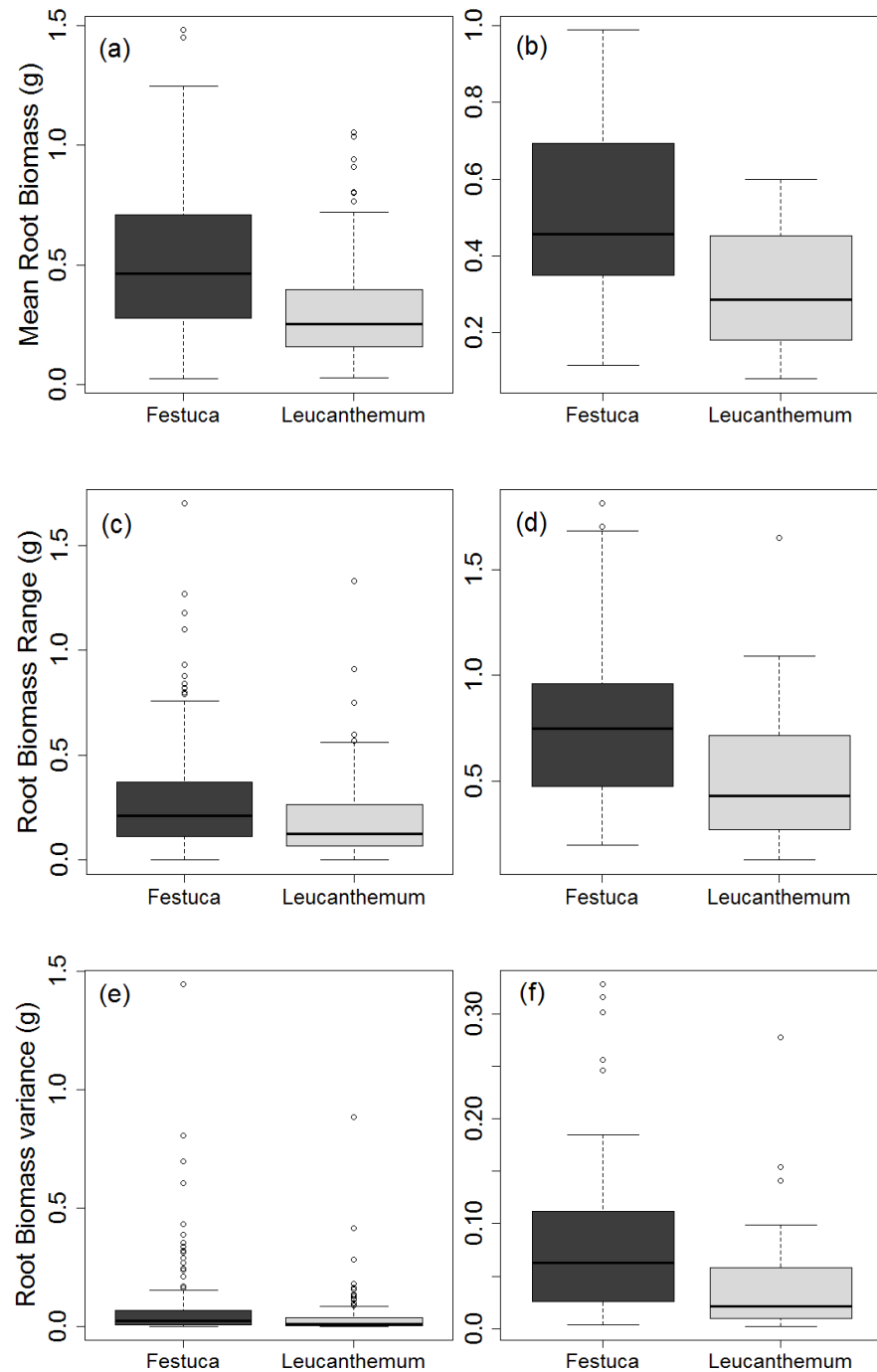


Figure 4.5. Root biomass mean, range and variance for *Festuca* and *Leucanthemum* soil cores. (a), (c) and (e) are data between neighbouring soil cores ( $n = 280$ ) and (b), (d) and (f) are data between the eight soil cores surrounding each plant ( $n = 70$ ).

#### 4.5.2.2 Scale-dependency of Root biomass heterogeneity

##### *Spatial autocorrelation of root biomass differs qualitatively and quantitatively in the AM fungal microhabitat surrounding F. rubra and L. vulgare*

The root biomass in the core-soil samples surrounding *F. rubra* was positively autocorrelated at small spatial scales (< 4 metres) and at around 20 metres (Fig 4.6 (a)), but was spatially uncorrelated at all other spatial scales. The root biomass in core-soil samples surrounding *L. vulgare* was considerably more positively spatially autocorrelated at small scales (<8 metres) than the *F. rubra* samples. However, the root biomass of the *L. vulgare* cores was significantly negatively spatially autocorrelated at scales of approximately 12 and 40 metres (Fig 4.6 (b)).

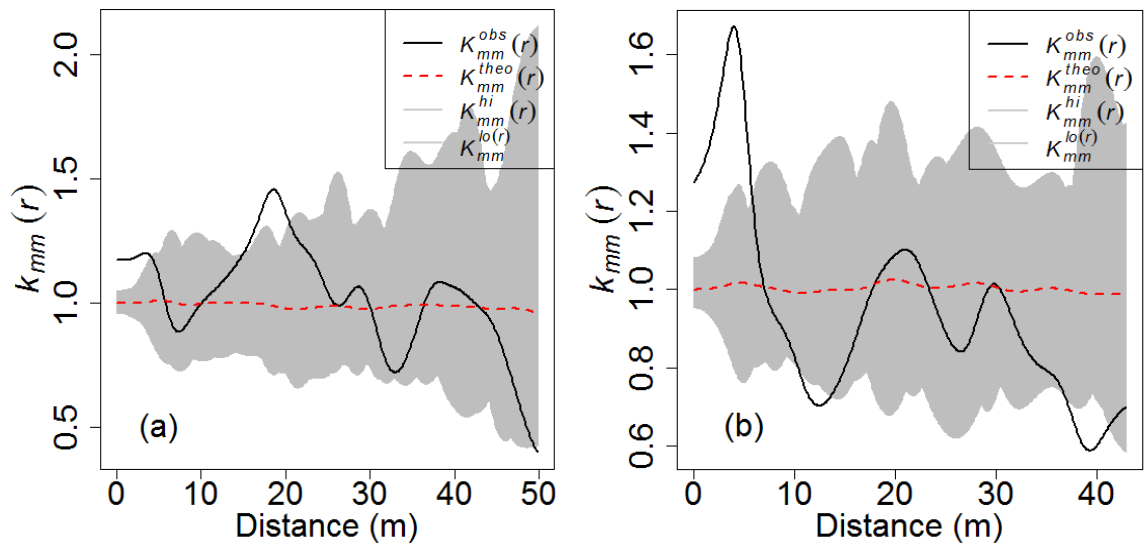


Figure 4.6. Correlograms of the mark correlation function for the biomass of roots in each soil core. (a) *F. rubra* cores (b) *L. vulgare* cores. Red dashed line represents a theoretical simulated population under Complete Spatial Randomness (CSR). Black line is the observed mark correlation function ( $K_{mm}(r)$ ). Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.

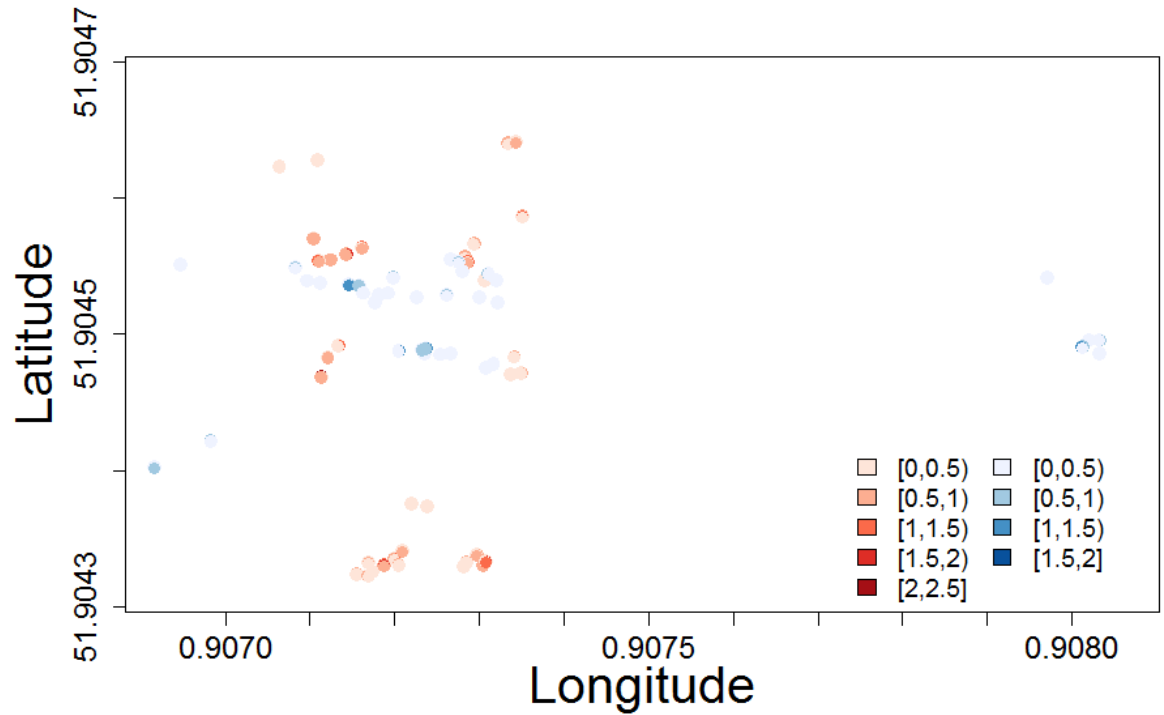


Figure 4.7. Root biomass heatmap. *F. rubra* (red) and *L. vulgare* (blue). Root biomass measured in grams.

#### 4.5.2.3 Bulk density is greater in the AM fungal habitat around *F. rubra*

Soil from the *Festuca* cores had significantly greater bulk density than the soil from the *Leucanthemum* cores when data from all cores were analysed separately ( $F_{1,558} = 91.3$ ,  $P < 0.0001$ ), when the means of neighbouring pairs were analysed ( $F_{1,278} = 57.8$ ,  $P < 0.0001$ ) (Fig. 4.8 (a)) and when the means of the eight cores surrounding each plant were analysed ( $F_{1,68} = 22.5$ ,  $P < 0.0001$ ) (Fig. 4.8 (b)). However, there was no significant difference in the range in bulk density between neighbouring pairs of cores between *Festuca* and *Leucanthemum* ( $F_{1,278} = 3.17$ ,  $P = 0.076$ ) (Fig. 4.8 (c)). There was no difference in the range in bulk density between the eight cores surrounding each plant ( $F_{1,68} = 1.73$ ,  $P = 0.19$ ) (Fig. 4.8 (d)). There was no difference between *Festuca* and *Leucanthemum* in the variance of the bulk density between

neighbouring pairs of cores ( $F_{1,278} = 3.6$ ,  $P = 0.059$ ) (Fig. 4.8 (e)), or in the variance for the eight cores surrounding each plant ( $F_{1,68} = 3.53$ ,  $P = 0.065$ ) (Fig. 4.8 (f)).

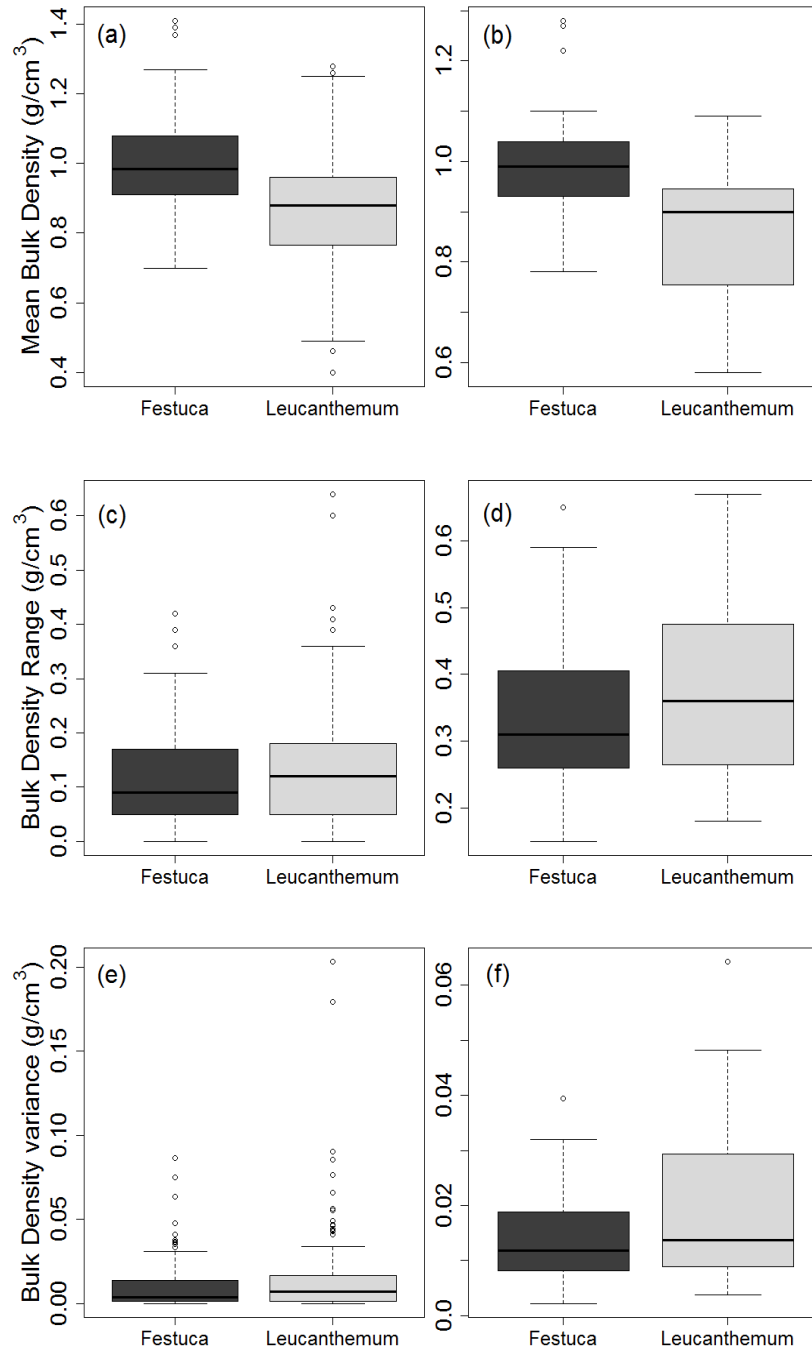


Figure 4.8. Bulk density mean, range and variance for *Festuca* and *Leucanthemum* soil cores. (a), (c) and (e) are data between neighbouring soil cores ( $n = 280$ ) and (b), (d) and (f) are data between the eight soil cores surrounding each plant ( $n = 70$ ).

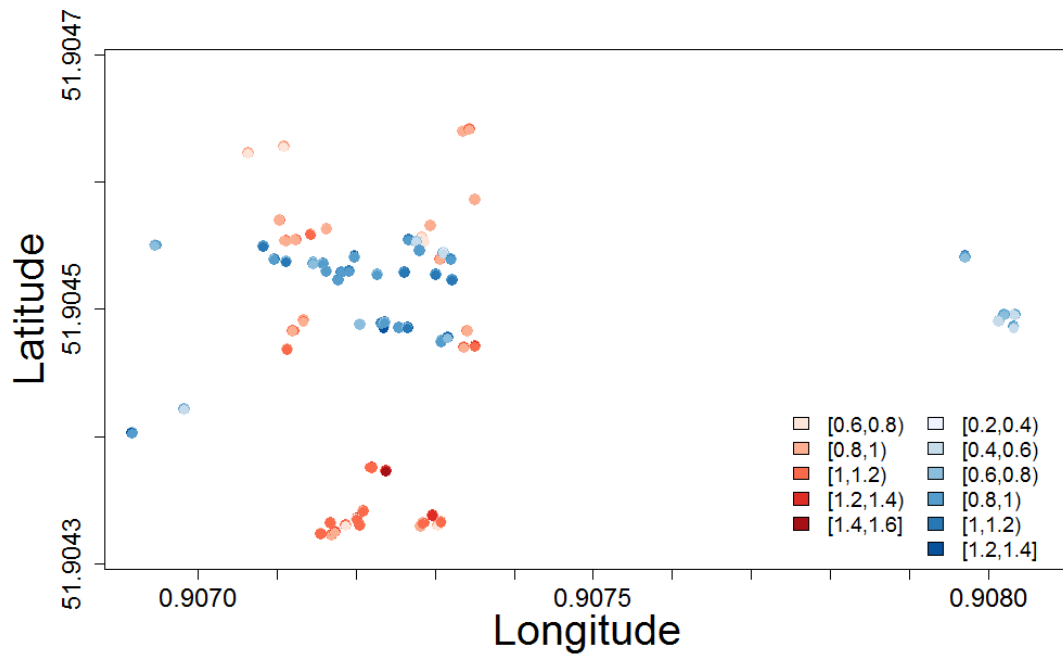


Figure 4.9. Bulk density heatmap. *F. rubra* (red) and *L. vulgare* (blue). Root biomass measured in grams per cm<sup>3</sup>.

#### 4.5.2.4 Scale-dependency of Bulk density heterogeneity

Spatial autocorrelation of bulk density differs qualitatively and quantitatively in the AM fungal microhabitat surrounding *F. rubra* and *L. vulgare*

The bulk density in the core-soil samples surrounding *F. rubra* was positively autocorrelated at scales of approximately 10m, 40m and 50m but was spatially uncorrelated at all other spatial scales (Fig. 4.10 (a)). The bulk density in core-soil samples surrounding *L. vulgare* was positively spatially autocorrelated at small scales (<4 m) and at large spatial scales (>35m), but, conversely to the *F. rubra* samples, negatively spatially autocorrelated at 10m (Fig. 4.10 (b)).

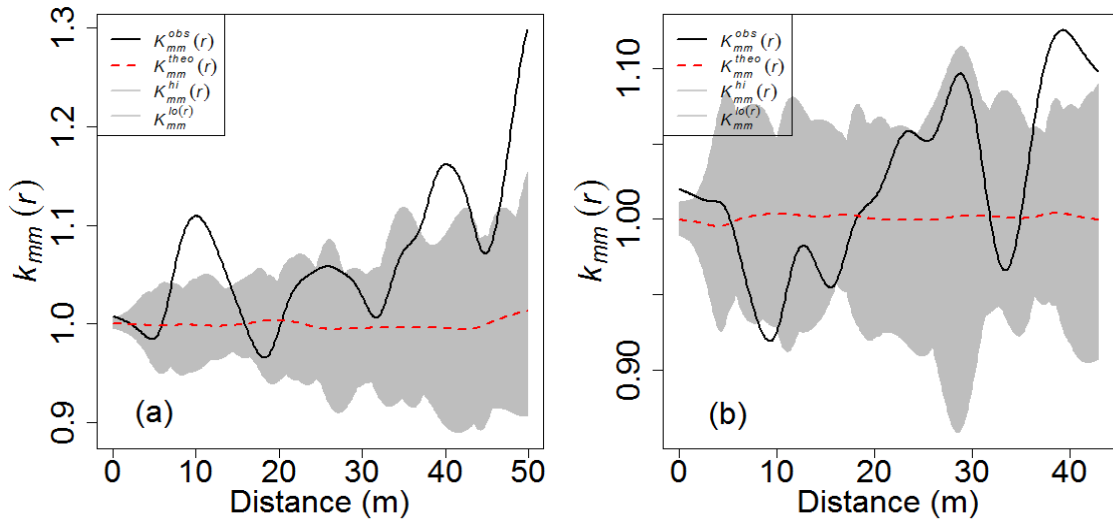


Figure 4.10. Correlograms of the mark correlation function for the dry bulk density of soil in each soil core. (a) *F. rubra* cores (b) *L. vulgare* cores. Red dashed line represents a theoretical simulated population under Complete Spatial Randomness (CSR). Black line is the observed mark correlation function ( $K_{mm}(r)$ ). Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.

#### 4.5.2.5 Total Organic Carbon is greater in the AM fungal habitat around *F. rubra*

The *F. rubra* core-soil samples had significantly greater mean TOC values than the *L. vulgare* soil samples when data from all cores were analysed separately ( $F_{1,557} = 7.4$ ,  $P = 0.007$ ) and when the means of neighbouring pairs were analysed ( $F_{1,277} = 4.3$ ,  $P = 0.039$ ) (Fig. 4.11 (a)). However, there was no difference in TOC content between the core-soil samples when the means of the eight cores surrounding each plant were analysed ( $F_{1,67} = 1.6$ ,  $P = 0.21$ ) (Fig. 4.11 (b)). There was no difference between *F. rubra* and *L. vulgare* in the range in TOC between neighbouring pairs of soil-core samples ( $F_{1,277} = 3.07$ ,  $P = 0.08$ ) (Fig. 4.11 (c)) or in the range in TOC between the eight cores surrounding each plant ( $F_{1,67} = 0.47$ ,  $P = 0.5$ ) (Fig. 4.11 (d)). The variance of the TOC between neighbouring pairs of core-soil samples was significantly greater in *L. vulgare* than in *F. rubra* ( $F_{1,277} = 4.13$ ,  $P = 0.043$ ) (Fig. 4.11 (e)), but there was no difference between host plant species in the variance of the TOC content for the eight cores surrounding each plant ( $F_{1,67} = 1.46$ ,  $P = 0.23$ ) (Fig. 4.11 (f)).

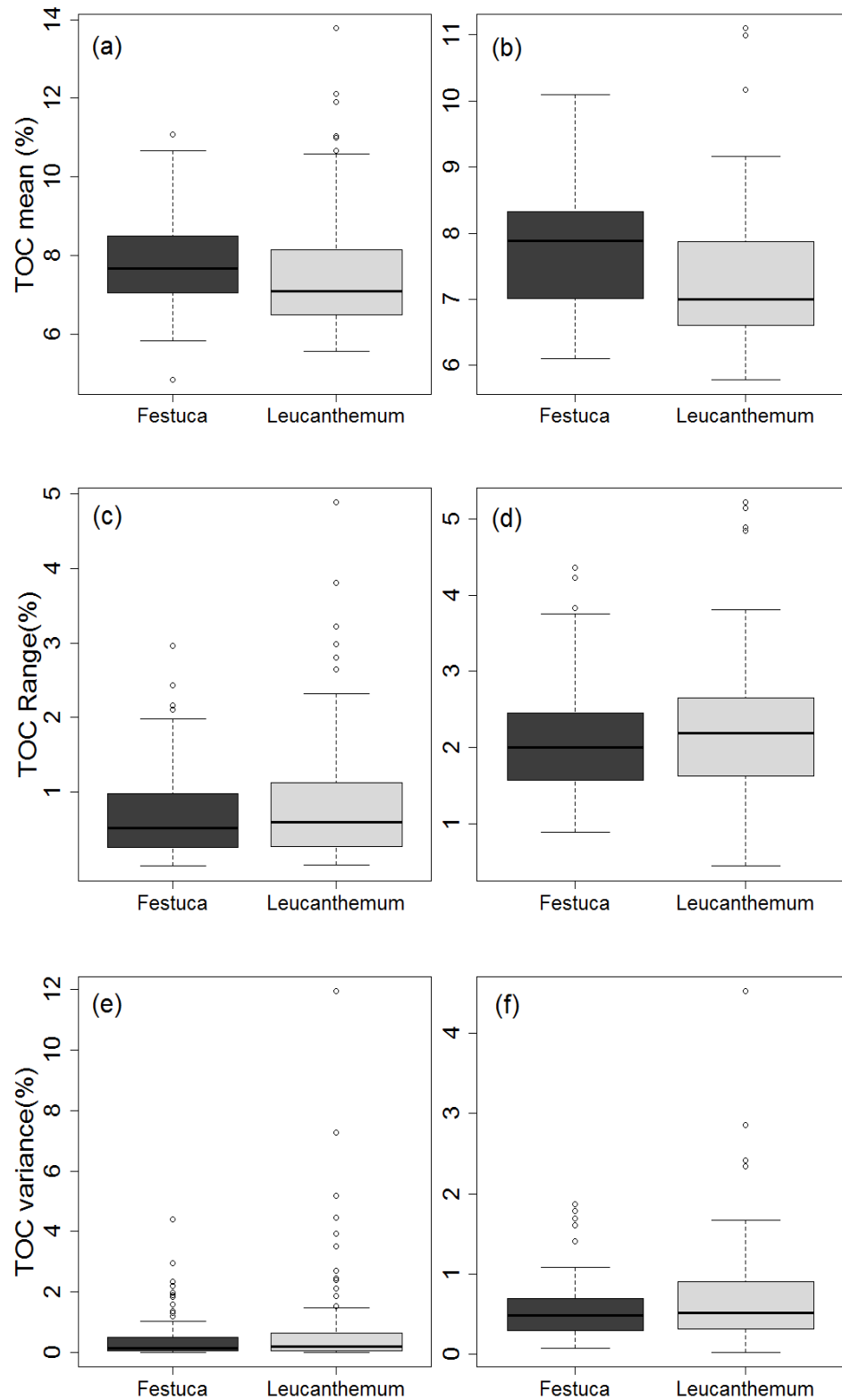


Figure 4.11. Total organic carbon mean, range and variance for *Festuca* and *Leucanthemum* soil cores. (a), (c) and (e) are data between neighbouring soil cores ( $n = 279$ ) and (b), (d) and (f) are data between the eight soil cores surrounding each plant ( $n = 70$ ). Data are only from the soil cores (excluding rhizosphere soil)



#### 4.5.2.6 Total Organic Carbon content of plant-soil samples

##### Mean values

The *F. rubra* soil samples had significantly greater mean TOC values than the *L. vulgare* soil samples when both plant-soil samples and core-soil samples were included in the analysis ( $F_{1,626} = 8.1$ ,  $P = 0.005$ ), when the means of neighbouring pairs of soil-core samples and plant-soil samples were analysed ( $F_{1,553} = 9.6$ ,  $P = 0.002$ ) (Fig. 4.12 (a)) and when the means of the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores were analysed ( $F_{1,274} = 5.5$ ,  $P = 0.02$ ) (Fig. 4.12 (b)). There was no difference between host plant species in the TOC content of the soil samples when means of the eight core-soil samples and the plant-soil sample were analysed ( $F_{1,67} = 1.7$ ,  $P = 0.19$ ) (Fig. 4.12 (c)).

##### Range values

There was no difference between host plant species in the range in TOC values between neighbouring pairs of soil samples when soil-core samples and plant-soil samples were analysed ( $F_{1,553} = 1.04$ ,  $P = 0.31$ ) (Fig. 4.12 (d)), when the range between the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores was analysed ( $F_{1,274} = 0.07$ ,  $P = 0.8$ ) (Fig. 4.12 (e)), or when the range between the core-soil samples and the plant-soil sample were analysed ( $F_{1,67} = 0.2$ ,  $P = 0.66$ ) (Fig. 4.12 (f)).

##### Variance values

Similarly, there was no difference between host plant species in the variance of TOC values between neighbouring pairs of soil samples when soil-core samples and

plant-soil samples were analysed ( $F_{1,553} = 1.6$ ,  $P = 0.21$ ) (Fig. 4.12 (g)), when the variance between the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores was analysed ( $F_{1,274} = 0.29$ ,  $P = 0.59$ ) (Fig. 4.12 (h)), or when the variance between the core-soil samples and the plant-soil sample were analysed ( $F_{1,67} = 1.01$ ,  $P = 0.32$ ) (Fig. 4.12 (i)).

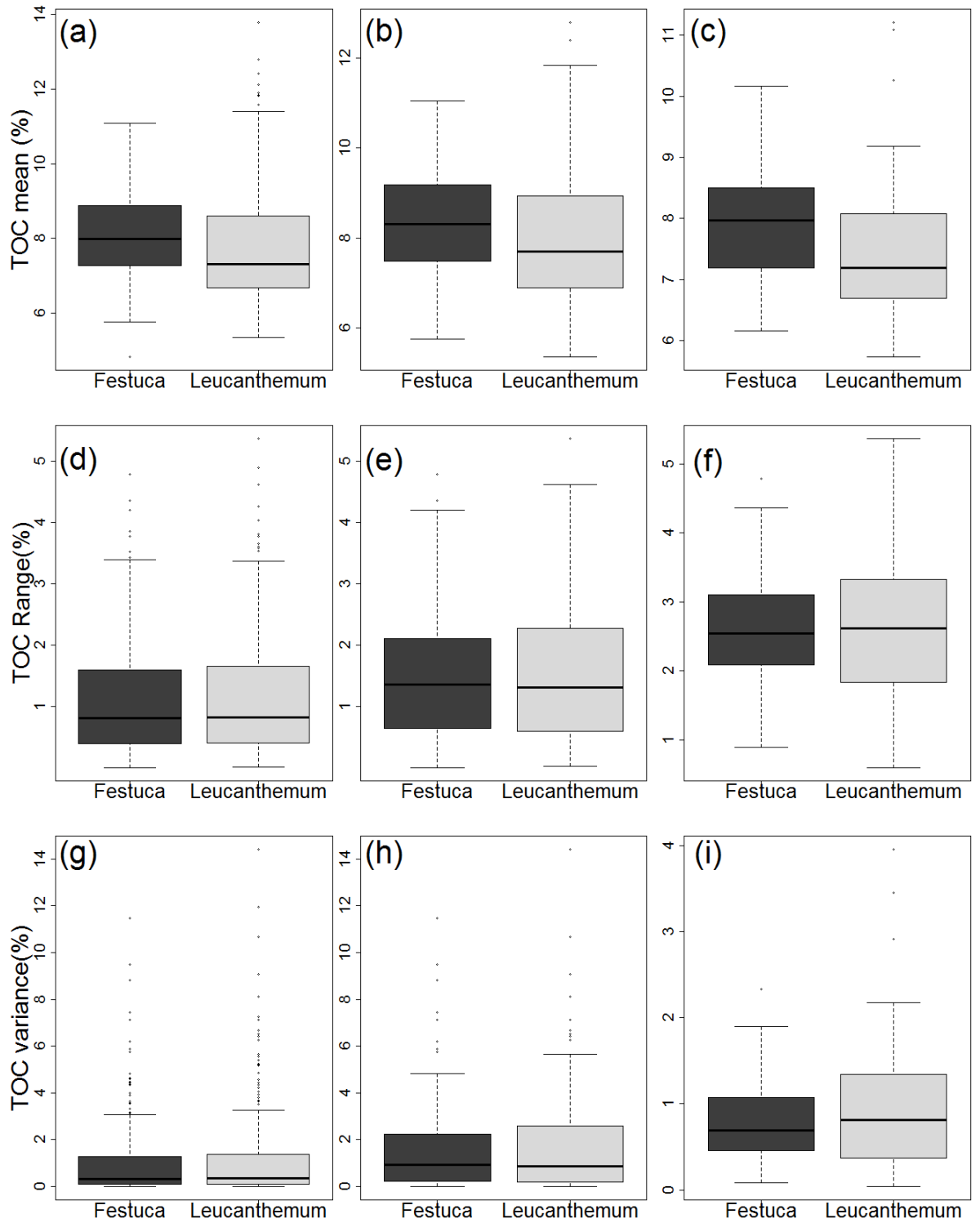


Figure 4.12. Total organic carbon mean, range and variance for *Festuca* and *Leucanthemum* soil cores. (a), (d) and (g) are data between neighbouring soil cores and the rhizosphere soil ( $n = 555$ ), (b), (e) and (h) are data between the plant-soil sample for each plant and its four neighbouring soil cores ( $n = 276$ ) and (c), (f) and (i) are data between the eight core-soil samples surrounding each plant and their corresponding plant-soil sample ( $n = 70$ ).

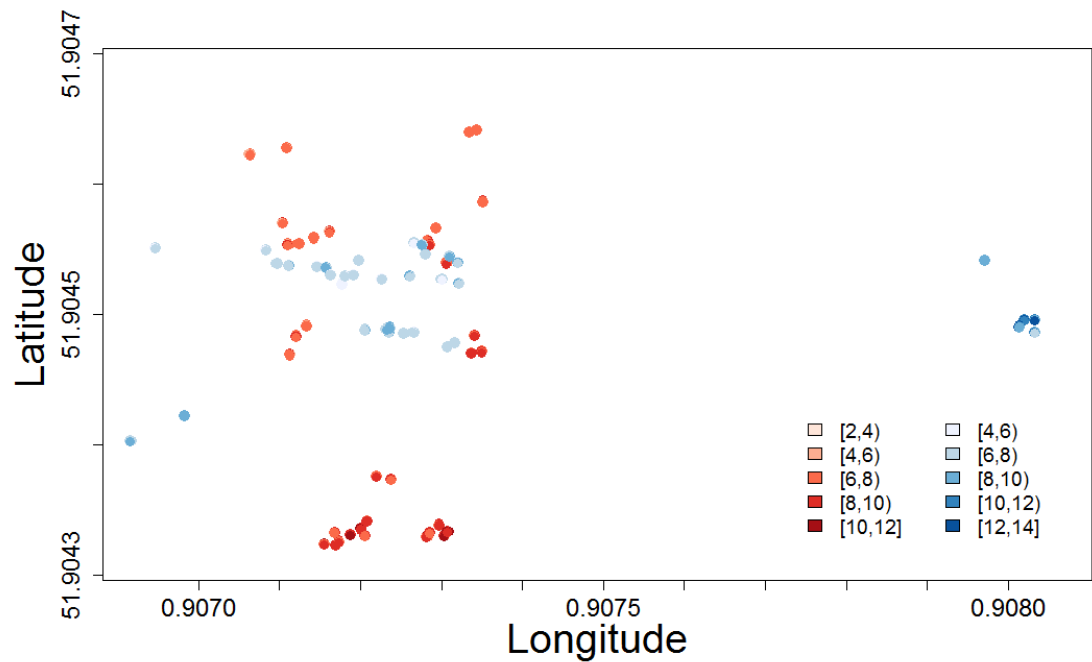


Figure 4.13. Heat map of TOC values for each soil core (Red = *Festuca rubra*, Blue = *Leucanthemum vulgare*). Values in percentages.

#### 4.5.2.7 Scale-dependency of TOC heterogeneity

Spatial autocorrelation of TOC differs qualitatively and quantitatively in the AM fungal microhabitat surrounding *F. rubra* and *L. vulgare*

The TOC in the core-soil samples surrounding *F. rubra* was positively autocorrelated at scales ranging from 0 - 25m but was spatially uncorrelated at all other spatial scales (Fig. 4.14 (a)). The TOC in core-soil samples surrounding *L. vulgare* was positively spatially autocorrelated at small scales (6 - 17m), but negatively spatially autocorrelated at large scales (18 - 44m) (Fig. 4.14 (b)).

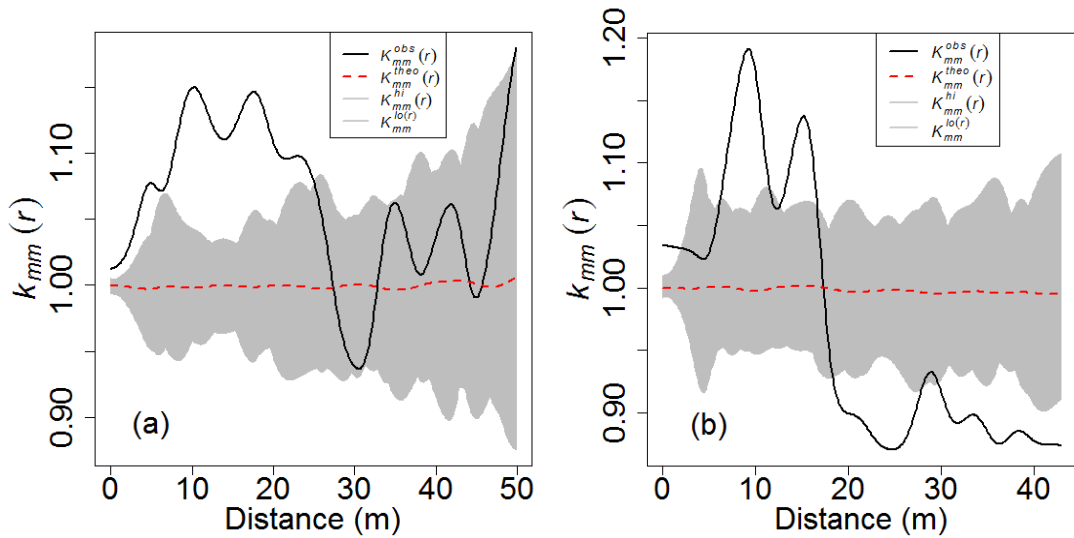


Figure 4.14. Correlograms of the mark correlation function for the Total organic carbon (TOC) content of soil in each soil core. (a) *F. rubra* cores (b) *L. vulgare* cores. Red dashed line represents a theoretical simulated population under Complete Spatial Randomness (CSR). Black line is the observed mark correlation function ( $K_{mm}(r)$ ). Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.

#### 4.5.2.8 Soil pH of core-soil samples

The *F. rubra* core-soil samples had significantly greater mean pH values than the *L. vulgare* soil samples the means of neighbouring pairs were analysed ( $F_{1,278} = 54.1$ ,  $P < 0.0001$ ) (Fig. 4.15 (a)) and when the means of the eight cores surrounding each plant were analysed ( $F_{1,68} = 19.9$ ,  $P < 0.0001$ ) (Fig. 4.15 (b)). There was no difference between *F. rubra* and *L. vulgare* in the range in pH between neighbouring pairs of soil-core samples ( $F_{1,278} = 0.24$ ,  $P = 0.62$ ) (Fig. 4.15 (c)) or in the range in TOC between the eight cores surrounding each plant ( $F_{1,68} = 0.009$ ,  $P = 0.93$ ) (Fig. 4.15 (d)). Similarly, there was no difference between the host plant species in the variance in pH between neighbouring pairs of soil-core samples ( $F_{1,278} = 0.42$ ,  $P = 0.52$ ) (Fig. 4.15 (e)) or in the variance in pH values between the eight cores surrounding each plant ( $F_{1,68} = 0.01$ ,  $P = 0.92$ ) (Fig. 4.15 (f)).

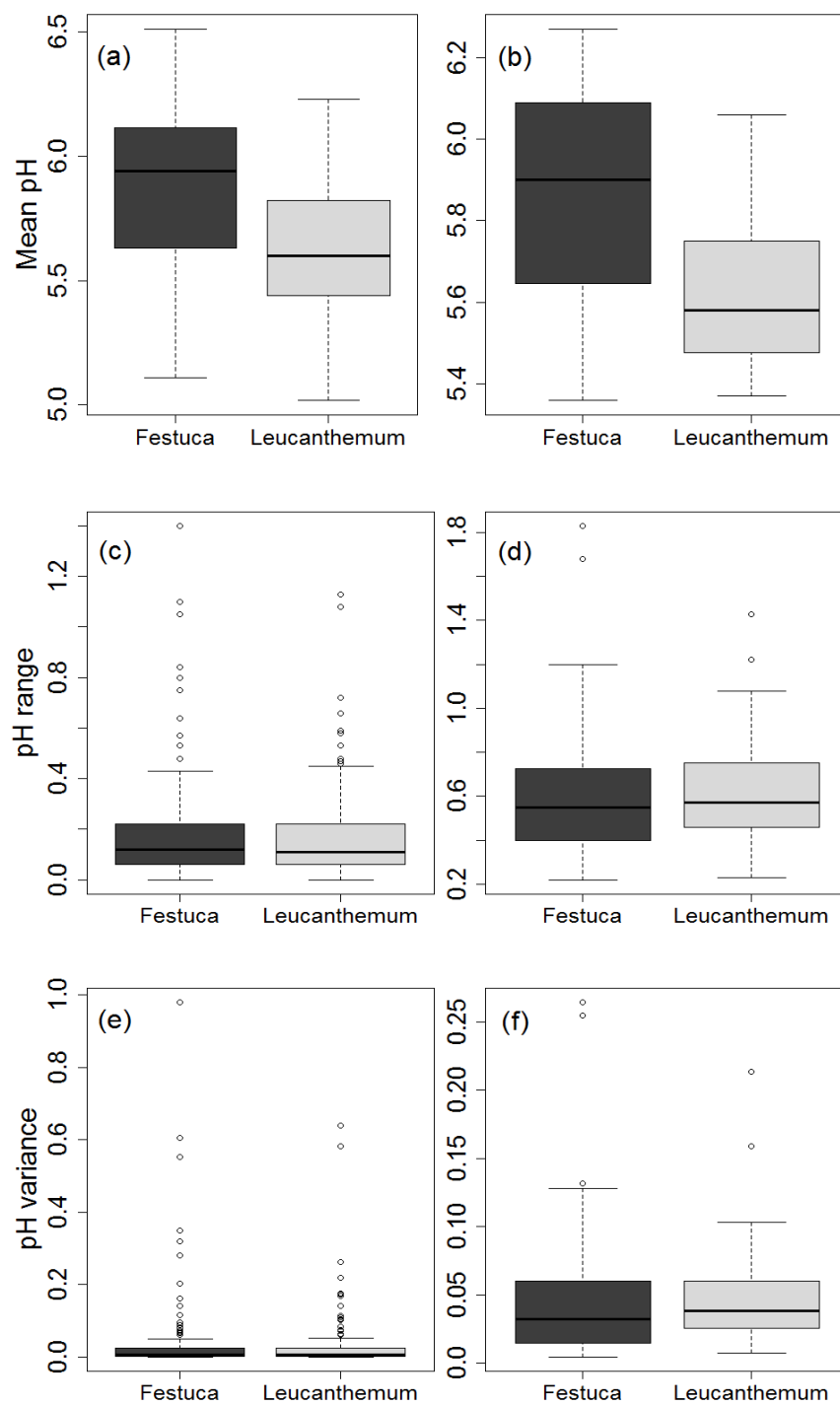


Figure 4.15. The mean, range and variance of pH values for *Festuca* and *Leucanthemum* soil cores. (a), (c) and (e) are data between neighbouring soil cores ( $n = 280$ ) and (b), (d) and (f) are data between the eight soil cores surrounding each plant ( $n = 70$ ). Data are only from the soil cores (excluding rhizosphere soil)

#### 4.5.2.9 Soil pH of plant-soil samples

##### Mean values

The *F. rubra* soil samples had significantly greater mean pH values than the *L. vulgare* soil samples when the means of neighbouring pairs of soil-core samples and plant-soil samples were analysed ( $F_{1,554} = 105$ ,  $P < 0.001$ ) (Fig. 4.16 (a)), when the means of the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores were analysed ( $F_{1,274} = 50.9$ ,  $P < 0.001$ ) (Fig. 4.16 (b)) and when means of the eight core-soil samples and the plant-soil sample were analysed ( $F_{1,68} = 19.2$ ,  $P < 0.001$ ) (Fig. 4.16 (c)).

##### Range values

There was no difference between host plant species in the range in pH between neighbouring pairs of soil samples when soil-core samples and plant-soil samples were analysed ( $F_{1,554} = 0.13$ ,  $P = 0.72$ ) (Fig. 4.16 (d)), when the range between the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores was analysed ( $F_{1,274} = 0.0$ ,  $P = 0.99$ ) (Fig. 4.16 (e)), or when the range between the core-soil samples and the plant-soil sample were analysed ( $F_{1,68} = 0.0$ ,  $P = 0.99$ ) (Fig. 4.16 (f)).

##### Variance values

Similarly, there was no difference between host plant species in the variance in pH values between neighbouring pairs of soil samples when soil-core samples and plant-soil samples were analysed ( $F_{1,554} = 0.68$ ,  $P = 0.41$ ) (Fig. 4.16 (g)), when the variance between the neighbouring pairs comprising the plant-soil sample for each plant and its four neighbouring soil cores was analysed ( $F_{1,274} = 0.26$ ,  $P = 0.61$ ) (Fig.

4.16 (h)), or when the variance between the core-soil samples and the plant-soil sample were analysed ( $F_{1,68} = 0.053$ ,  $P = 0.82$ ) (Fig. 4.16 (i)).

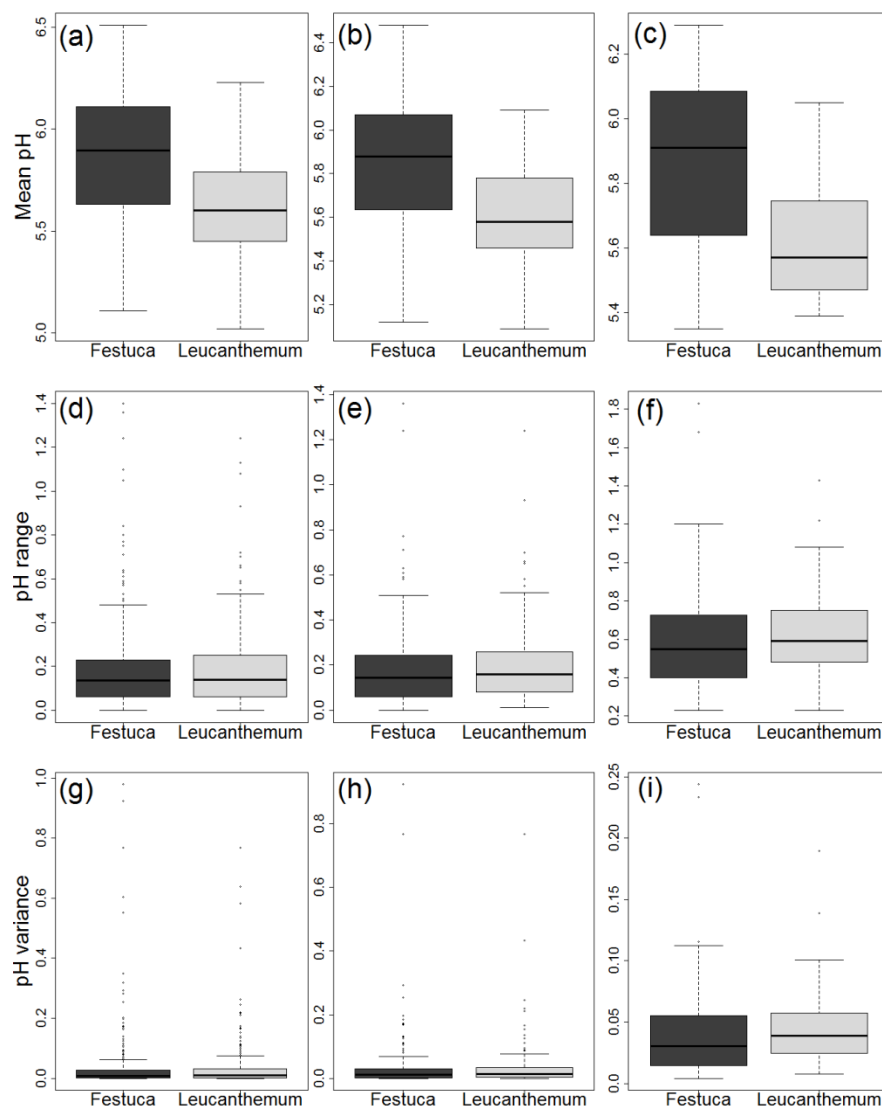


Figure 4.16. pH mean, range and variance for *Festuca* and *Leucanthemum* soil cores. (a), (d) and (g) are data between neighbouring soil cores and the rhizosphere soil ( $n = 555$ ), (b), (e) and (h) are data between the plant-soil sample for each plant and its four neighbouring soil cores ( $n = 276$ ) and (c), (f) and (i) are data between the eight core-soil samples surrounding each plant and their corresponding plant-soil sample ( $n = 70$ ).



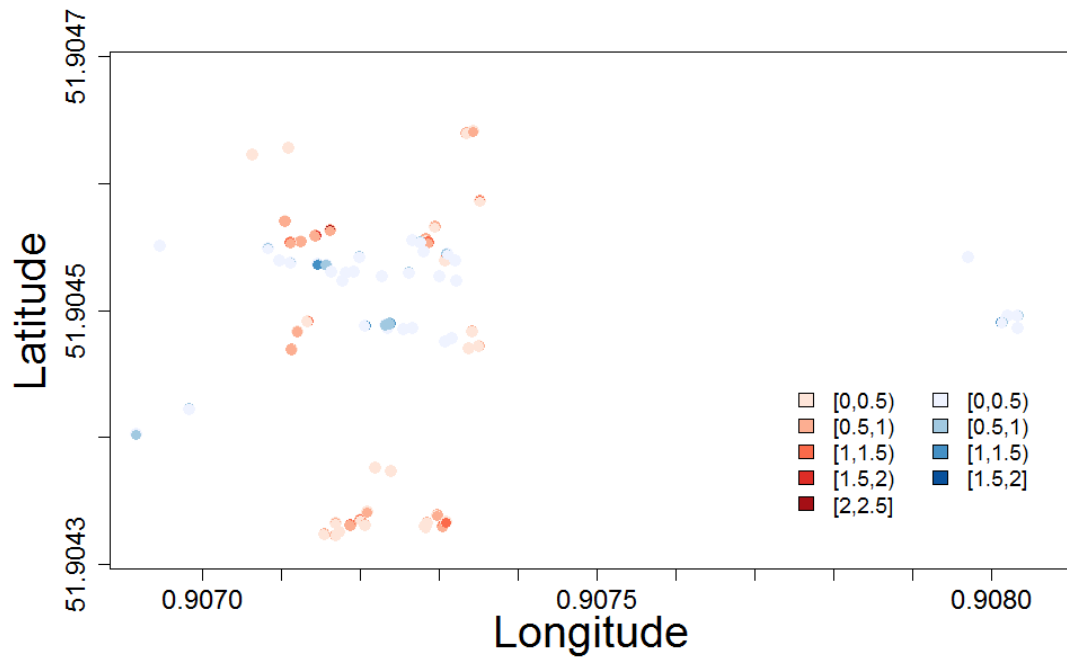


Figure 4.17. Heat map of pH values for each soil core (Red = *Festuca rubra*, Blue = *Leucanthemum vulgare*).

#### 4.5.2.10 Scale-dependency of pH heterogeneity

*Spatial autocorrelation of pH differs qualitatively and quantitatively in the AM fungal microhabitat surrounding F. rubra and L. vulgare*

The pH in the core-soil samples surrounding *F. rubra* was positively autocorrelated at scales ranging from 9 - 19m and at approximately 38m but was spatially uncorrelated at all other spatial scales (Fig. 4.18 (a)). The pH in core-soil samples surrounding *L. vulgare* was positively spatially autocorrelated at the large scale (33m), but negatively spatially autocorrelated at small scales (5 and 10m) (Fig. 4.18 (b)).

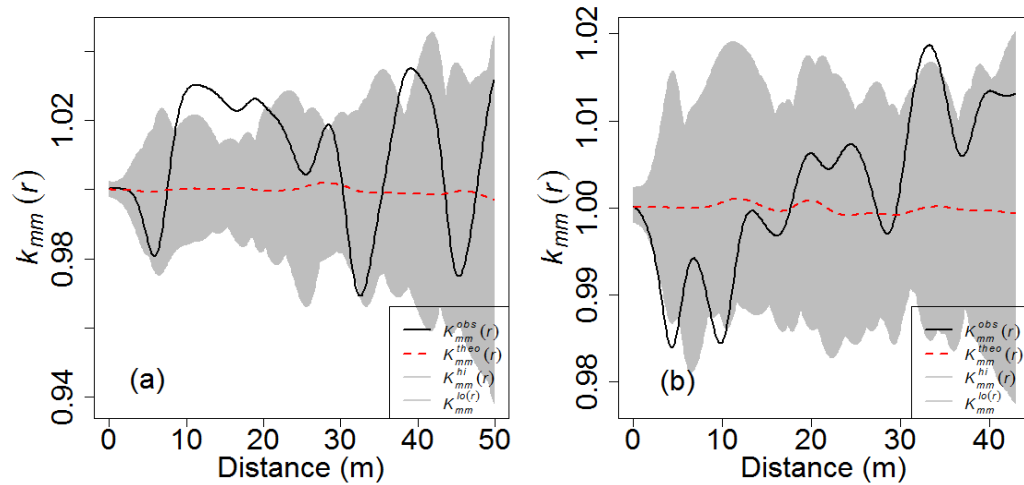


Figure 4.18. Correlograms of the mark correlation function for the pH of soil in each soil core. (a) *F. rubra* cores (b) *L. vulgare* cores. Red dashed line represents a theoretical simulated population under Complete Spatial Randomness (CSR). Black line is the observed mark correlation function ( $K_{mm}(r)$ ). Grey envelope indicates confidence values for a community operating under CSR, generated by 99 simulations.

#### 4.5.3 Molecular data

The molecular AM fungal community dataset to be sequenced on the MiSeq platform was not yet available at the time of writing, due to global problems with the Illumina 600-cycle kits.

## 4.6 Discussion

### 4.6.1 Different host plant species provide their AM fungal communities with different plant and soil-based microhabitats

Differences between the two host plants *F. rubra* and *L. vulgare* were detected in every one of the soil environmental variables tested in this study. The soil surrounding *F. rubra* plants had greater root biomass, bulk density, TOC and pH values than that surrounding *L. vulgare*. Furthermore, the root biomass, and consequently, the root:shoot ratio, of the *F. rubra* plants themselves were greater than that of *L. vulgare*. Given the absolute dependence of AM fungi on plant roots, this difference in root biomass in the rhizosphere soil and surrounding soil,

represents a difference in the amount of available AM fungal habitat. The greater availability of habitat to *F. rubra*-associated AM fungi than to *L. vulgare*-associated AM fungi likely contributes to both the greater species richness and the lower diversity index of AM fungal communities associated with *F. rubra* (chapter 2). More species can coexist but the communities may be dominated by a single taxon, due to priority effects favouring early colonisers (chapters 3 & 5). This is contrary to the finding that species-poor communities tend to be more uneven (chapter 3). Profiling the AM fungal communities at a wide range of spatial scales, as well as measuring other important soil environmental variables, could determine whether this disagreement is due to spatial scale of sampling. The greater density of roots around *F. rubra* could even compensate for the greater bulk density of the soil, which might otherwise render the habitat less suitable for certain AM fungal species, whose hyphal growth rates and biomass allocation may make them less likely to predominate in soils of a greater bulk density (Dodd *et al.*, 2000, Hart and Reader, 2002). The observed greater values of TOC and pH in the *Festuca* soils could also be explained by the greater root density. A greater biomass of roots increases the biological activity in the rhizosphere, in terms of rhizophagous and mycophagous grazing, by providing the soil environment with more energy and potentially a greater degree of heterogeneity. This would lead to increased biomass and eventually greater organic carbon levels within the soil. Similarly, if plant roots increase soil pH by absorbing nitrogen as  $\text{NO}_3^-$  (Nye, 1981), the more roots there are, the greater the pH values will be. Regardless of the mechanisms involved, these differences in soil parameters between plant species represent significant variation in the niche of AM fungi.

#### 4.6.2 Differences between host plant species in TOC content of surrounding soil are influenced by spatial scale of sampling

The total organic carbon (TOC) content of soil was the only one of the four soil environmental variables in the study whose qualitative difference between host plant species was dependent upon spatial scale. At the smallest spatial scale, core-soil samples of *F. rubra* had greater TOC content than those of *L. vulgare*, but at the larger spatial scale, there was no difference. In the case of all other soil environmental variables, the qualitative differences between host plant species did not depend on spatial scale. This is likely due to the greater biomass of roots in *F. rubra* plants increasing the biological activity, eventually leading to greater TOC content of the soil at the smallest spatial scale (5cm between soil cores). The larger spatial scale (20cm around each plant) inevitably incorporates a greater root biomass from surrounding plants, regardless of the host plant species. In a grassland ecosystem with a community dominated by the same few plant species, a maximum density of plant roots is probably attained at this larger scale, resulting in no difference in TOC content of soils between *F. rubra* and *L. vulgare*. The amount of TOC in soils can influence AM fungal hyphal growth either positively or negatively (Joner and Jakobsen, 1995, Ravnskov *et al.*, 1999), so the scale dependency of this soil property is likely to affect the distribution of AM fungal extraradical mycelium. Lekberg *et al.* (2007) observed that TOC was one of the most important factors in the partitioning of niche space in the Glomeraceae, along with total N, with which it was more closely correlated than either pH, nitrate, total P, soil moisture or percentage clay.

#### 4.6.3 The degree of environmental heterogeneity differs between host plant species at large, but not small, spatial scales

Neither the range nor the variance values for each of the soil environmental variables differed between plant species at the two smallest spatial scales (5cm and 20cm). This result is confirmed by the similar values of the mark correlation function,  $K_{mm}(r)$ , for *F. rubra* and *L. vulgare* at the smallest spatial scale (Figs. 4.6, 4.10, 4.14 & 4.18). At larger spatial scales (>3m), however, the type and degree of spatial autocorrelation of values for each of the four environmental variables differs between host plant species. There is a negative relationship between AM fungal colonisation of host plant roots and environmental heterogeneity (chapter 5), possibly because habitats with greater heterogeneity incorporate more environments that are less suitable for AM fungal colonisation. Similarly, a negative relationship between heterogeneity in root availability and AM fungal species richness also exists (chapter 2). A clear difference between the two host plant species in this study is that there is a much greater degree of negative spatial autocorrelation in the values of root biomass, bulk density, TOC and pH in the soil samples from the *L. vulgare* environment. The greater degree of environmental heterogeneity resulting from this negative spatial autocorrelation could be sufficient to influence the AM fungal community structure and diversity at large spatial scales. The compositional divergence of AM fungal communities in different host plant species as spatial scale increases (chapter 2) could contribute to the result that host plant preference is detected more often at large scales (Hazard *et al.*, 2013) than at small scales (Santos *et al.*, 2006). The relatively long-term continuity of the grassland used in this study (>9 years since disturbance) and the rhizomatous nature of *F. rubra* and *L. vulgare* have resulted in the two species occupying similar proportions of their

potential niche at the small spatial scale. If two plant species differ in their dispersal ability, then a disparity between the proportions of their potential niches that each plant is occupying will become evident. In this case, the heterogeneity to which the host plants subject their associated AM fungal communities will differ. If, on the other hand, they do not differ in their dispersal ability and the ranges of environmental variables they can tolerate are also similar, then the proportion of their potential niches which they occupy will not differ (Thorhallsdottir, 1990). The dispersal ability of *F. rubra* and *L. vulgare* may differ only at these larger spatial scales, and as a result, the heterogeneity of the habitat of each plant differs at larger scales. Alternatively, *L. vulgare* may be able to tolerate a wider range in absolute values of certain soil parameters, which may only be detectable at larger spatial scales when a greater degree of heterogeneity is incorporated into the study area. This may provide the findings of Öpik *et al.*(2009) with a potential mechanism for their observation that specialist AM fungi are more likely to associate with habitat specialist host plant species and generalist AM fungi more likely to associate with generalist host plant species. The host plant and the fungal partner may co-occur due to similar ranges of environmental variables they can tolerate.

#### **4.6.4 Experimental limitations and further work**

So inextricable are the soil variables that affect the distribution of AM fungi, that resolving the causal mechanisms is a complex process. The further use of observational studies, and of manipulative experimental studies, could be valuable in achieving this aim. For instance, the measurement of soil environmental variables over a range of spatial scales, such as was done in the current study, over a greater temporal range in the same habitat could help to determine how the environmental heterogeneity potentially perceivable by AM fungi correlates with time since

disturbance in a natural environment. Multifactorial experimental studies could control for multiple factors that influence AM fungal communities simultaneously, and therefore determine the pathways through which certain physical properties of the soil and the host plant are mediated.

#### **4.6.5 Conclusions**

AM fungi inhabiting different host plants are subject to differing environmental parameters, both biotic and abiotic. The complexity and variability of these parameters, which comprise the environmental heterogeneity to which AM fungi are subject, only differ between host plant species at large spatial scales, potentially influencing the scale dependence of AM fungal community diversity, composition and structure, and the perceived host plant preference. The interspecific differences in certain important soil physical properties, in this case the total organic carbon content of the soil, are only significant at certain spatial scales. As such, host plant-specific effects on associated AM fungal communities may also only be observable at certain spatial scales.

## Chapter 5: Effects of environmental heterogeneity and energy availability on arbuscular mycorrhizal assemblages

### 5.1 Summary

- The individual and synergistic effects of energy availability and environmental heterogeneity on AM fungal community diversity, structure and competition is poorly understood. The manner in which energy affects the influence of heterogeneity on communities has implications for carbon sequestration in soils and the maintenance of AM fungal, hence plant, biodiversity.
- To determine the effects of energy availability and environmental heterogeneity, a multifactorial experimental design subjected AM fungal communities associated with *Brachypodium sylvaticum* to varying levels of energy and heterogeneity, in the form of varying levels of light intensity and soil water content, respectively.
- Plant physical properties were more influenced by energy availability and AM fungal root colonisation was more influenced by environmental heterogeneity. Greater availability of energy only had a significant effect on AM fungal root colonisation in the highest heterogeneity treatment, suggesting suppression of carbon allocation to AM fungi in highly heterogeneous environment with a low availability of energy.

### 5.2 Introduction

A central goal in community ecology is to identify the factors that affect variation in the diversity, composition and structure of communities and to quantify their relative influence. Two environmental properties which have been extensively studied with



this aim in mind are environmental heterogeneity and energy availability (productivity). Patterns of biodiversity along gradients of heterogeneity and productivity have historically been most widely studied in macroorganisms (Fischer, 1960, Pianka, 1966). Only relatively recently have these fundamental determinants of community structure and function been studied in microbes (Horner-Devine *et al.*, 2003, Ramette and Tiedje, 2007, Mohamed and Martiny, 2011). Given the importance of microbes to ecosystem functioning in every ecosystem, this represents a significant shortfall in ecology. The negative relationship between AM fungal species richness and heterogeneity that has been observed (chapter 2), the potential for overdominance by a single AM fungal taxon to decrease community diversity (chapter 3) and the spatial variability in environmental heterogeneity (chapter 4) all suggest that the availability of energy and the heterogeneity of the habitat have a substantial effect on AM fungal communities. The synergistic effects of energy and heterogeneity on AM fungal community dynamics are likely to be considerable and complex.

#### *5.2.1 Heterogeneity effects on microbial diversity*

Reported effects of heterogeneity on microbial diversity vary widely. In an experimental study using populations of different morphotypes of the bacterium *Pseudomonas fluorescens*, Brockhurst *et al.* (2007) observed that a greater niche occupation within a community is more likely to inhibit the initial diversification of an invading morphotype. The authors of this study admit, however, that this reductionist approach represents a simple ecosystem with only one trophic level, and that in the more complex ecosystems in nature, greater diversity could provide more ecological niches and thus promote greater diversification. Niches linked to parasitism, predation and host-specific mutualistic relationships are perhaps among the most

prominent that could increase in number as a result of greater species diversity. Indeed, in a natural environment, Ramette and Tiedje (2007) reported that the genetic diversity of the soil-borne bacterium *Burkholderia ambifara* increased as soil environmental heterogeneity increased. The patterns of community composition and structure varied at the small scales at which the environmental heterogeneity was perceivable. The effect of soil heterogeneity on arbuscular mycorrhizal fungal communities has a relatively short history (Camargo-Ricalde and Esperón-Rodríguez, 2005, Whitcomb and Stutz, 2007). Camargo-Ricalde and Esperón-Rodríguez (2005) reported greater numbers of spores in soils with a greater degree of spatial heterogeneity in the soil, but the effect on spore diversity is not clear. The structure, diversity and composition of AM fungal communities are highly influenced by soil physical properties (Dumbrell *et al.*, 2010b, Hazard *et al.*, 2013). Hence environments with greater environmental heterogeneity should support a greater AMF diversity. Indeed, a greater AM fungal biovolume such as that observed in the study by Camargo-Ricalde and Esperón-Rodríguez (2005) has been linked to increased diversity (Antoninka *et al.*, 2011). However, sometimes the spatial structure of AM fungi has been observed to be largely independent from spatial heterogeneity. Whitcomb and Stutz (2007) assessed AM fungal diversity on two 84m<sup>2</sup> experimental plots to test for effects of environmental heterogeneity. They found that the AM fungal species diversity only showed spatial structure at the smallest spatial scale of the study (at which they were negatively autocorrelated) and at larger scales, all species recorded apart from two were randomly spatially distributed. Inevitably, effects of heterogeneity depend on the variable selected for quantification of heterogeneity. For instance, while greater heterogeneity in soil pH has been linked to increased AM fungal species richness (Dumbrell *et al.*, 2010b),

the reverse is true for heterogeneity in habitat availability (Chapter 2). As AM fungal habitat availability is dependent on a suite of other environmental factors, a negative heterogeneity-diversity relationship is likely for those factors that affect the availability of habitat. For those factors that do not significantly affect the availability of habitat, a unimodal, hump-shaped relationship is likely. This is because highly heterogeneous environments can incorporate habitats near or outside the limits of the AM fungal niche, limiting diversity, and environments of a low heterogeneity, if they are optimal for AM fungal survival and growth could result in a few species dominating the community. Determining the heterogeneity-diversity relationship for a range of environmental variables for AM fungi could inform land management practice to maximise AM fungal biodiversity.

#### 5.2.2 Energy effects on microbial diversity

Horner-Devine *et al.* (2003) observed different responses to a productivity gradient in different bacterial taxa within the same system. A U-shaped relationship was observed in the  $\alpha$ -proteobacteria, whereas the Cytophaga-Flavobacterium-Bacteroides (CFB) group and algae both showed an opposite, hump-shaped relationship between productivity and diversity. The  $\beta$ -proteobacteria showed no significant relationship. One explanation the authors posed for the co-existence of hump-shaped and U-shaped relationships within the same system is that competition between the two taxa impedes high levels of diversity of both taxa. Scheiner and Jones (2002) suggest that the U-shaped productivity-diversity relationship could, at certain, larger spatial scales, be an artefact of the selection of unusually species-rich communities at either end of the productivity gradient. This would result in the communities at intermediate productivity levels to have lower species diversity than either of the extremes, producing the U-shaped relationship.

This is more likely to be the case when the U-shaped relationship is observed in isolation, but in a situation such as that described by Horner-Devine *et al.* (2003), competition between taxa seems to be the more parsimonious explanation. Not only have microbes displayed positive relationships between diversity and a direct measure of productivity, but they have also been observed to vary in diversity along gradients of latitude, disturbance, climate and salinity (Mohamed and Martiny, 2011). Each of these physical properties of a system, however, is likely to covary with the amount of available energy. The majority of any effect of available energy on AM fungal community diversity and structure is likely to be mediated by their plant hosts, given that the fungal partner relies completely on phytogenic carbon for its energy supply (Helgason and Fitter, 2009). Indeed, CO<sub>2</sub> enrichment experiments show that not only can greater energy availability increase AM fungal biomass and community diversity (Sanders *et al.*, 1998, Antoninka *et al.*, 2011) but it can also alter community composition (Cotton *et al.*, 2015). In systems with a greater availability of energy, the allocation of carbon to AM fungi will be greater, and greater biovolume will result, potentially resulting in greater diversity. The positive feedback mechanism which affords a carbon allocation benefit to the first AM fungal coloniser of a plant root (Helgason and Fitter, 2009) may cause a negative energy-diversity relationship at the upper end of the spectrum of energy availability. This may result in a unimodal, hump shaped relationship between energy and AM fungal species richness.

### 5.2.3 Interaction of energy and heterogeneity

Unsurprisingly, the synergistic effects of environmental heterogeneity and available energy on diversity have been more extensively studied in macro-organisms than in

micro-organisms. Ruggiero and Kitzberger (2004) conducted a study examining the effects of habitat heterogeneity and the availability of energy within a system on the species richness of eight taxa of South American mammals. Their analysis confirmed that patterns of variation in mammal species richness at the continental scale are explained by the positive synergistic effects between energy availability and heterogeneity. They observed three ways in which energy availability (measured as normalised difference vegetation index, NDVI) and environmental heterogeneity can interact to influence diversity in a positive way (Fig. 5.1). Heterogeneity had little to no effect on species richness in the orders Edentata and Chiroptera. An additive effect was observed in rodents of the infraorder Hystricognathi and the Primates, wherein species richness increases in response to increased heterogeneity and increased energy availability, but their effects are independent of one another. A pattern in which heterogeneity-richness relationships become steeper as energy availability increases was observed in the Carnivora, Artiodactyla, Sciurognathi rodents and the Marsupialia (Fig. 5.1). While multiple energy-heterogeneity-diversity patterns were observed in these Mammalian taxa, by no means were all possible relationships represented. No U-shaped, Hump-shaped or negative energy-diversity relationships were recorded. Neither were negative energy-diversity relationships accounted for. If similar methodologies were implemented in microbial ecology, many more patterns describing the synergistic effects of heterogeneity and energy on diversity would inevitably be observed. Indeed, given the importance of microbes in all known ecosystems, determining how they are influenced by energy and heterogeneity should be a priority. AM fungi energy-heterogeneity-diversity patterns are likely to take many forms, including a cone-shaped relationship, in which intermediate levels of heterogeneity and energy result in the highest diversity. This is

most likely to be the case when the variable whose heterogeneity is being measured does not affect the availability of AM fungal habitat (root biomass). For those variables that do significantly influence the availability of habitat, such as soil bulk density and water content, a ridge-shaped pattern, in which intermediate levels of energy and low levels of heterogeneity produce the highest levels of diversity, is likely.

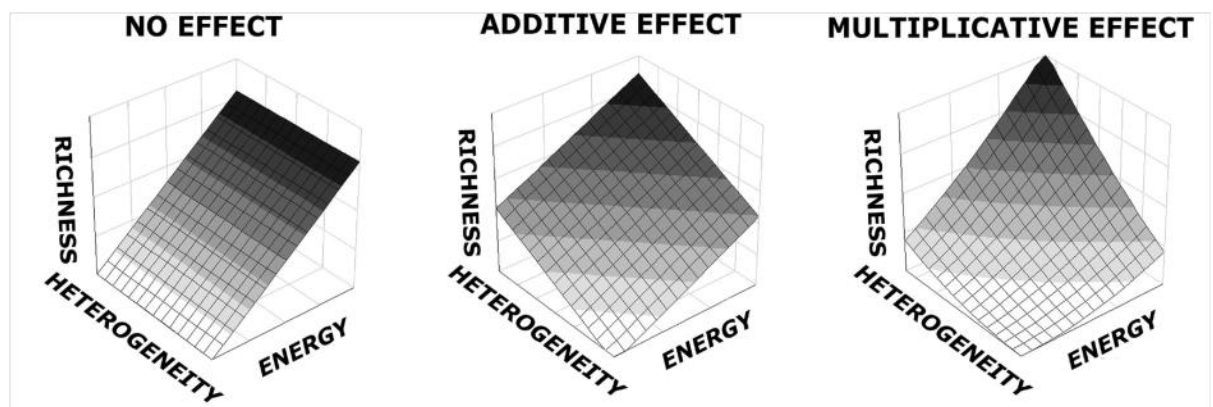


Figure 5.1. Synergistic effects on species richness between habitat heterogeneity and energy availability. Species richness is mainly controlled by energy availability (no effect of heterogeneity; left), Habitat heterogeneity and energy availability contribute independently to species richness (additive effects; centre) and the effects of heterogeneity increase in importance as energy availability increases (Multiplicative effect, right). From: Ruggiero and Kitzberger (2004).

### 5.3 Aims

In this study the following hypotheses are being tested:

1. Gradients in energy and heterogeneity affect the phytogenic biotic variables to which AM fungal communities are subject
2. There is a positive relationship between available energy and AM fungal biomass
3. There is a negative relationship between environmental heterogeneity generated by soil water content gradient and AM fungal biomass

4. AM fungal community diversity, composition and structure are affected by both available energy and heterogeneity

## 5.4 Materials and Methods

### 5.4.1 Plant Growth experiment



Figure 5.2 *Brachypodium sylvaticum* seedlings in the growth cabinet

#### 5.4.1.1 Growth medium preparation

Soil inoculum from unmowed grassland habitat in Colchester, UK (See chapter 4 for details) was sieved with a 2mm soil sieve. To dilute the soil and encourage root colonisation by AM fungi, sharp sand (Homebase, UK) was dried, sieved (2mm) and mixed with the soil inoculum in a 2:1 ratio. An equal volume of soil/sand mixture was added to each pot (diameter 8cm). The soil in the pots was dried in a growth cabinet at 18°C and 0% humidity for 48 hours to evaporate the water from the soil. The pots were weighed and then placed in trays filled with water until the soil was fully saturated. The pots were weighed again and the weight at which each pot needed to

be maintained throughout the experiment was calculated based on the percentage saturation of the treatment group each pot was in.

#### *5.4.1.2 Heterogeneity and energy treatments*

Pots were grouped into three heterogeneity treatments (High, Intermediate and Low) and three energy treatments (High, Medium and Low). The heterogeneity treatments differed in the range of soil water content values they incorporated. Soil water content was selected as the variable to be manipulated to generate a heterogeneity gradient because of its considerable effects on AM fungal community diversity and composition (König *et al.*, 2010, Hawkes *et al.*, 2011, Kivlin *et al.*, 2011). The pots in the high heterogeneity group ranged from 40% to 100% saturation, the pots in the intermediate heterogeneity group ranged from 60% to 90% saturation and the pots in the low heterogeneity group were all at 70% saturation (Tables 5.1 & 5.2). The three energy availability treatments (High, Medium and Low), hereafter referred to as energy treatments, differed in the light intensity to which each group was subject (1.4.1.2). Each of the three heterogeneity treatments was subject to every energy treatment, resulting in a multifactorial experimental design such that the AM fungal communities associated with the host plants were subject to every possible combination of heterogeneity and energy treatments (Tables 5.1 & 5.2). This allows determination of the relative importance and the synergistic effects of energy availability and environmental heterogeneity on AM fungal colonisation rates, community diversity, structure and composition, as well as on host plant physical characteristics. Three replicates per pot resulted in 108 plants in the experiment. The roots of the four plants per energy-heterogeneity treatment (Table 5.1) were pooled to form one AM fungal community (Table 5.2).



Table 5.1. Multifactorial experimental design consisting of three heterogeneity treatments and three light intensity (energy) treatments. Numbers indicate percentage saturation of soil per pot, and letters denote low (L), medium (M) or high (H) light intensity.

		HETEROGENEITY		
		High	Intermediate	Low
Light Intensity	Low	L40	L60	L70
	Low	L60	L70	L70
	Low	L80	L80	L70
	Low	L100	L90	L70
	Medium	M40	M60	M70
	Medium	M60	M70	M70
	Medium	M80	M80	M70
	Medium	M100	M90	M70
	High	H40	H60	H70
	High	H60	H70	H70
	High	H80	H80	H70
	High	H100	H90	H70

Table 5.2. Multifactorial experimental design consisting of three heterogeneity treatments and three light intensity (energy) treatments, pooled into 9 AM fungal communities. Energy/heterogeneity treatment of each community denoted by XX.X, in which HH = High heterogeneity, IH = Intermediate heterogeneity, LH = Low heterogeneity, L = Low light intensity, M = Medium light intensity and H = High light intensity.

		HETEROGENEITY		
		High	Intermediate	Low
Light Intensity	Low	HH.L	IH.L	LH.L
	Medium	HH.M	IH.M	LH.M
	High	HH.H	IH.H	LH.H

#### 5.4.1.3 Germination and growth

*Brachypodium sylvaticum* seeds (Emorsgate seeds, Norfolk, UK) were stratified at 4°C in the dark, covered in sterile distilled water, for one week to minimise asynchrony in germination. This plant species was chosen because it is native to the

UK, highly mycorrhizal (Abeyakoon and Pigott, 1975), has a short generation time (Steinwand *et al.*, 2013) and is tolerant of a wide range in soil water content (Evans and Etherington, 1990). One plant was grown in each pot to ensure AM fungal communities within each plant were subjected to known, constant soil water content treatment, after which selective pooling of communities generates communities subject to varying levels of heterogeneity.

#### 5.4.1.4 Light regimes

To manipulate the energy available to the AM fungal communities, three light regimes were established in the growth chamber using varying numbers of layers of neutral density screening (Lee filters, Hampshire, UK). Light intensity was measured using an LI-250 Light meter (LI-COR, Lincoln, NE, USA). A mean light intensity value was obtained using five readings (one in the centre and one from each of the four corners) from each of the three blocks: High light intensity, medium light intensity and low light intensity (Table 3). As there is a positive relationship between the rate of carbon assimilation in plants and carbon allocation from the host plant to AM fungi (Lekberg *et al.*, 2013), these light regimes represent different levels of available energy to the AM fungal communities.

Table 5.3. Light intensity values for the three Light treatments ( $\mu\text{mol}/\text{sec}/\text{m}^2$ )

	High	Medium	Low
Mean	240.9	127.46	55.68
sd	32.29	17.24	5.64

#### 5.4.1.5 Growth and harvest

Plants were grown in a growth cabinet (Sanyo, Moriguchi, Japan) on a 15:9 hour day:night cycle (day temperature: 18°C, night temperature: 14°C, humidity: 70%) for

24 days, and watered to a constant weight every 48 hours, after which they were harvested. Soil was washed from the roots and the plants were weighed to an accuracy of 0.01 g. Plants were dried in a forced air oven at 70°C for 72 hours.

#### *5.4.2 Molecular methods*

The washed, dried fine roots from each plant were pooled according to the multifactorial experimental design in tables 5.1 and 5.2 (For each of the three light intensity treatments there were three heterogeneity treatments, each consisting of four watering treatments. Three replicates per watering treatment) were homogenised using stainless steel beads in microcentrifuge tubes on a TissueLyser II (Qiagen Ltd, W Sussex, UK). DNA was extracted from the twenty-seven root samples harvested from the plant growth experiment (three replicates of nine treatments, Table 5.2) using MoBio PowerPlant DNA isolation kit following the manufacturer's instructions (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Subsequent methods for the Next-Generation Sequencing of AM fungal communities were as described in chapter 4.

#### *5.4.3 Data analysis*

Comparisons of plant physical properties and AM fungal nucleic acid abundance between treatments were done using analysis of variance and Tukey's multiple comparison of means procedure in the stats package of the R statistical language (R-Development-Core-Team, 2011).

### *Gel quantification analysis*

Nucleic acid abundance was quantified for each sample by image analysis of Agarose gel images, using ImageJ (Rasband, 1997). This was used as a proxy for intraradical AM fungal biomass.

## **5.5 Results**

### *5.5.1 Plant data*

*Availability of energy, but not heterogeneity, affects the phytogenic biotic variables to which AM fungal communities are subject*

A two-way analysis of variance found that root weight differed significantly between Light treatments ( $F_{2,18} = 7.83$ ,  $P = 0.0036$ ), but not between Heterogeneity treatments ( $F_{2,18} = 0.31$ ,  $P = 0.74$ ). There was no interaction effect on root weight between light and heterogeneity ( $F_{4,18} = 0.19$ ,  $P = 0.94$ ) (Table 5.4, Fig. 5.3).

Table 5.4. Mean and standard deviation of root biomass values for each light treatment and heterogeneity treatment

	Mean	Standard deviation
<b>Light Level</b>		
High	0.042	0.017
Medium	0.036	0.015
Low	0.016	0.003
<b>Heterogeneity</b>		
High	0.031	0.016
Intermediate	0.034	0.019
Low	0.028	0.018

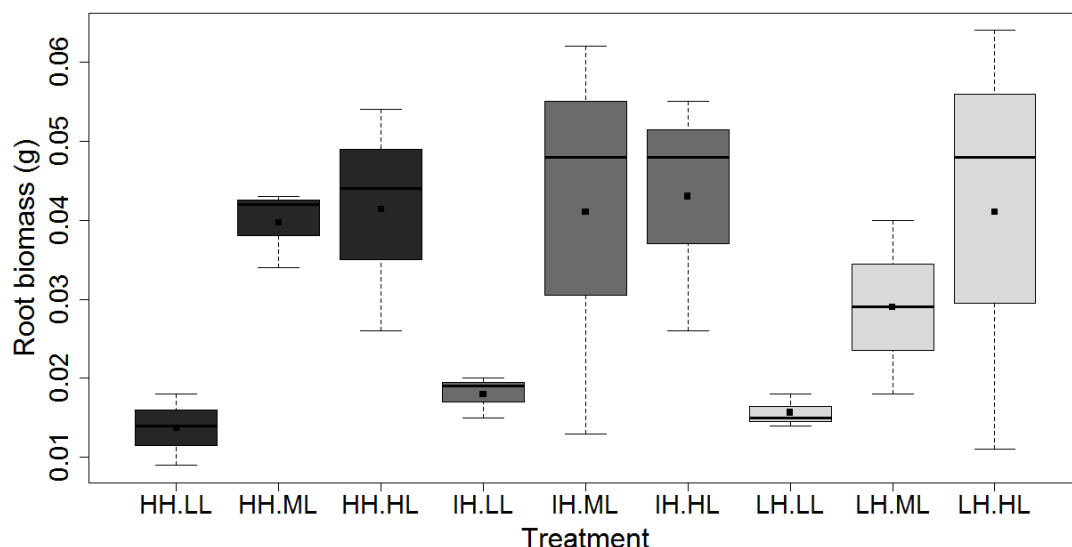


Figure 5.3. Total root biomass from each treatment. HH = High heterogeneity, IH = Intermediate heterogeneity, LH = Low Heterogeneity, HL = High Light intensity, ML = Medium Light intensity, LL = Low light intensity. See methods for details. Black squares are means.

The dry weight of the whole plant (roots + shoots) differed between the three light regimes in the experiment ( $F_{2,108} = 9.945$ ,  $P < 0.001$ ). A Tukey's multiple comparison of means procedure revealed that the whole plant dry weight of those plants under

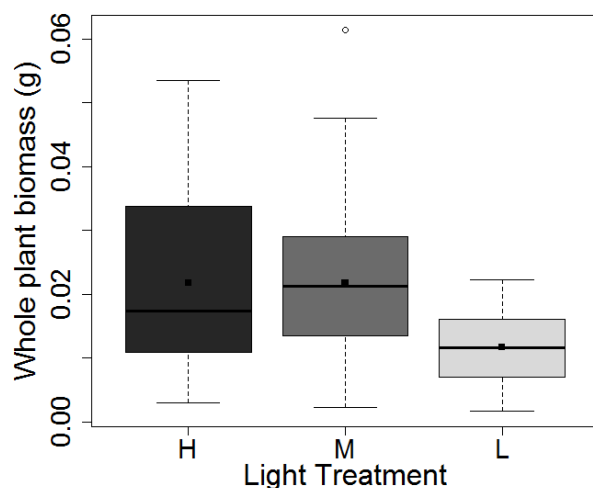


Figure 5.4. Whole plant Biomass for each light treatment. H = High Light intensity, M = Medium Light intensity, L = Low Light intensity. Black squares are means.

low light intensity differ from those under High light intensity and Medium light intensity ( $P < 0.001$ ), but those under medium light intensity and high light intensity do not significantly differ from each other ( $P = 1$ ) (Fig. 5.4).

The Root:Shoot ratio differed between the light regimes (Fig. 5.5) ( $F_{2,108} = 18.07$ ,  $P < 0.0001$ ). Tukey's multiple comparison of means procedure revealed that each pairwise comparison was significantly different: Low - High: ( $P < 0.0001$ ), Medium - Low: ( $P = 0.012$ ), Medium - High: ( $P = 0.007$ ).

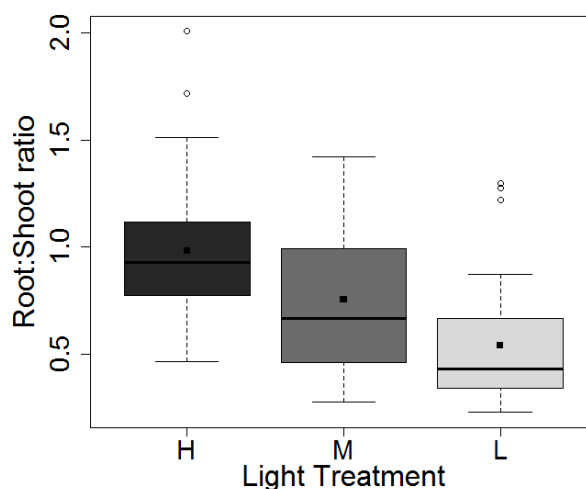


Figure 5.5. Root:Shoot ratio for each light treatment. H = High Light intensity, M = Medium Light intensity, L = Low Light intensity. Black squares are means.

The root dry weight differed between light regimes (Fig. 5.6) ( $F_{2,108} = 12.53$ ,  $P < 0.001$ ). A Tukey's multiple comparison of means procedure revealed that the root

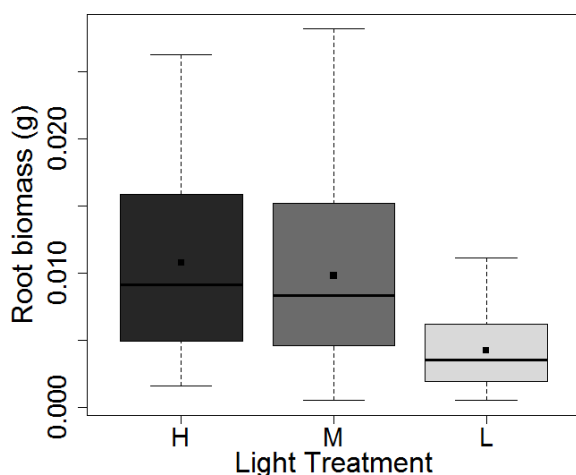


Figure 5.6. Root Biomass for each light treatment. H = High Light intensity, M = Medium Light intensity, L = Low Light intensity. Black squares are means.

dry weight of those plants under low light intensity differ from those under High light intensity and Medium light intensity ( $P < 0.001$ ), but those under medium light intensity and high light intensity do not significantly differ from each other ( $P = 0.77$ ).

Shoot dry weight differed between light regimes (Fig. 5.7) ( $F_{2,108} = 6.433$ ,  $P = 0.002$ ).

A Tukey's multiple comparison of means procedure revealed that shoot dry weight of those plants under low light intensity differ from those under High light intensity and Medium light intensity ( $P = 0.02$ ,  $P = 0.003$  respectively), but those under medium

light intensity and high light intensity do not significantly differ from each other ( $P = 0.74$ ).

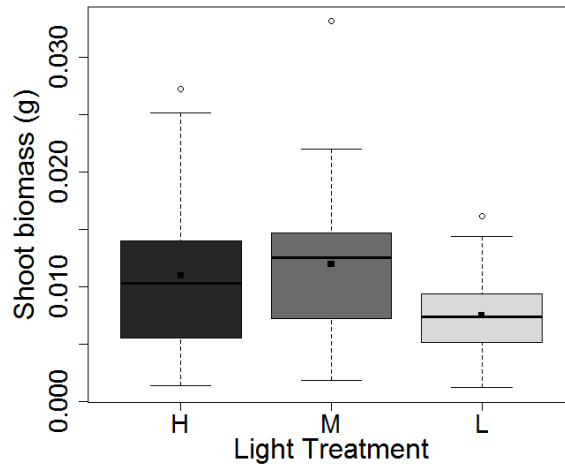


Figure 5.7. Shoot Biomass for each light treatment. H = High Light intensity, M = Medium Light intensity, L = Low Light intensity. Black squares are means.

There was no difference in dry weight of the whole plant between the watering regimes (Fig. 5.8) ( $F_{5,105} = 1.522$ ,  $P = 0.19$ ).

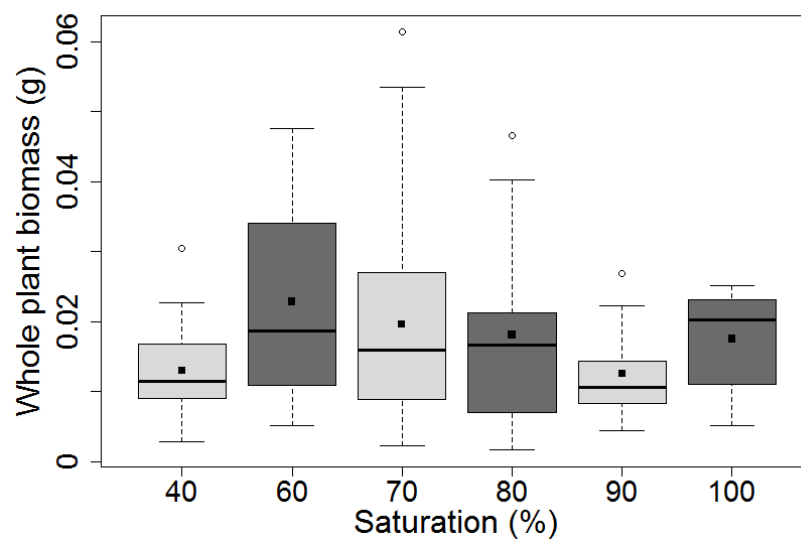


Figure 5.8. Whole plant Biomass for each watering regime. Black squares are means.

There was no difference in shoot dry weight between watering regimes (Fig. 5.9) ( $F_{5,105} = 1.464$ ,  $P = 0.21$ ).

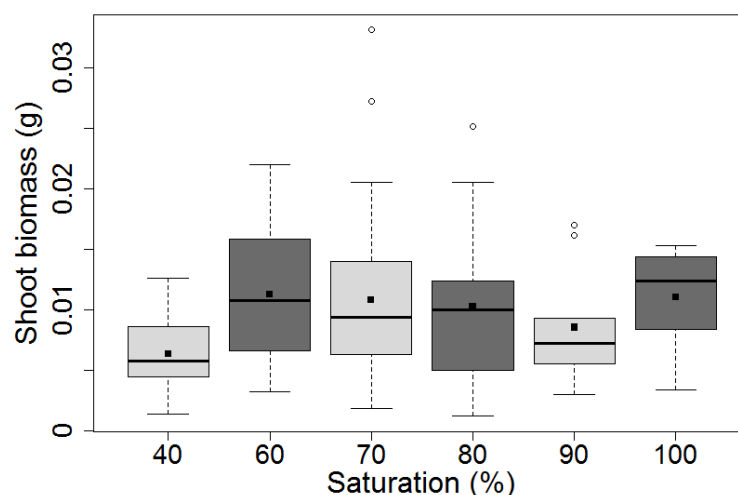


Figure 5.9. Shoot Biomass for each watering regime. Black squares are means.

There was no difference in root dry weight between watering regimes (Fig. 5.10)

( $F_{5,105} = 2.055$ ,  $P = 0.077$ ).

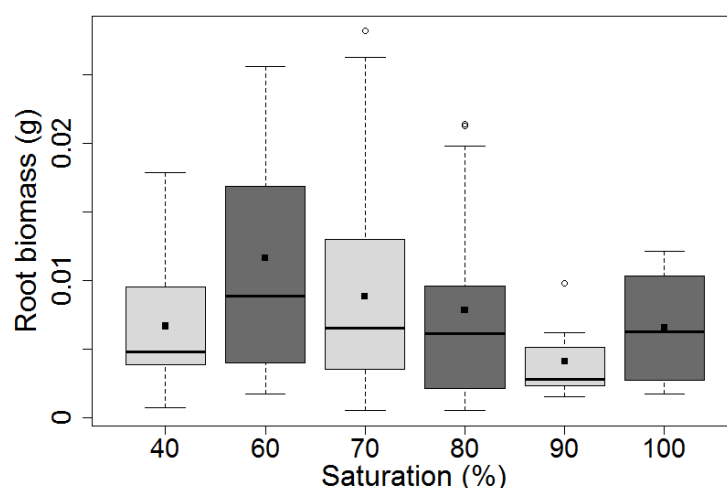


Figure 5.10. Root Biomass for each watering regime. Black squares are means.

The root:shoot ratio was different between the watering regimes (Fig. 5.11) ( $F_{5,105} = 4.21$ ,  $P = 0.0016$ ). A Tukey's multiple comparison of means procedure revealed that the root:shoot ratio of those plants watered to 40% saturation differed from those under 90% and 100% saturation ( $P = 0.009$ ,  $P = 0.04$  respectively). Those under 60% saturation differed from those under 90% saturation ( $P = 0.016$ ). Additionally, the difference between the root:shoot ratio of those plants under 60% saturation and



those under 100% saturation was approaching a 95% confidence level of significance ( $P=0.07$ ).

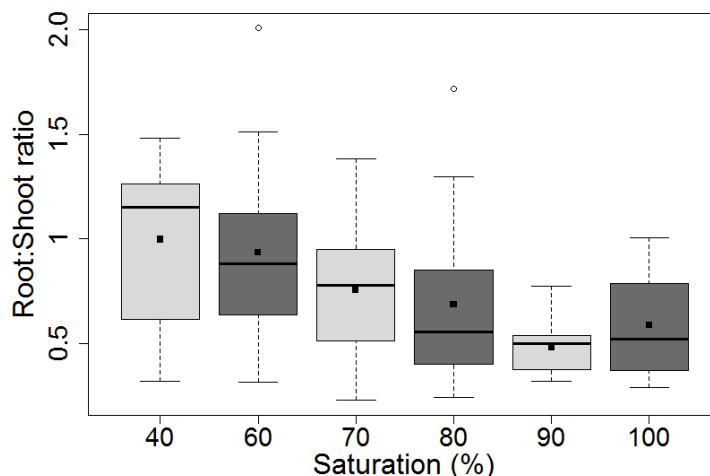


Figure 5.11. Root:Shoot ratio for each watering regime. Black squares are means.

## 5.5.2 AM fungal data

### 5.5.2.1 Heterogeneity has a greater effect on intraradical AM fungal biomass than energy availability

A two-way analysis of variance found that intraradical AMF biomass (for which the relative intensities of bands on the Agarose gel (Fig. 5.13) were a surrogate) differed significantly between heterogeneity treatments ( $F_{2,18} = 4.63$ ,  $P = 0.024$ ), but not between energy treatments ( $F_{2,18} = 1.84$ ,  $P = 0.19$ ) (Fig. 5.12). There was no interaction effect on AMF biomass between light and heterogeneity ( $F_{4,18} = 0.23$ ,  $P = 0.92$ ). AM fungal biomass was greatest under low heterogeneity treatments (Table 5.1, Fig. 5.12). And, while not significant, AM fungal biomass tended to be greater under high light intensity (Fig. 5.12). A Tukey's multiple comparison of means procedure on the two-way analysis of variance revealed that while there was no difference in band intensity between energy treatments, the low heterogeneity

treatment had a greater band intensity than either the intermediate heterogeneity ( $P=0.04$ ) or the high heterogeneity ( $P=0.045$ ) treatments.

#### 5.5.2.2 Availability of energy has a greater effect on intraradical AM fungal biomass in habitats with a high degree of heterogeneity

Comparisons between light treatments within heterogeneity treatments were tested with a one-way ANOVA separately. There was a significant difference between light treatments within the High Heterogeneity treatment ( $F_{2,6} = 13.8$ ,  $P = 0.006$ ). While there was no difference between the medium light and the low light treatments ( $P = 0.59$ ), the high light treatment had a greater band intensity than either the medium light ( $P = 0.018$ ) or the low light ( $P = 0.006$ ) treatments. No difference was detected between light treatments within either the Intermediate heterogeneity ( $F_{2,6} = 0.49$ ,  $P = 0.64$ ) or the Low heterogeneity ( $F_{2,6} = 0.49$ ,  $P = 0.63$ ) treatments (Fig. 5.12).

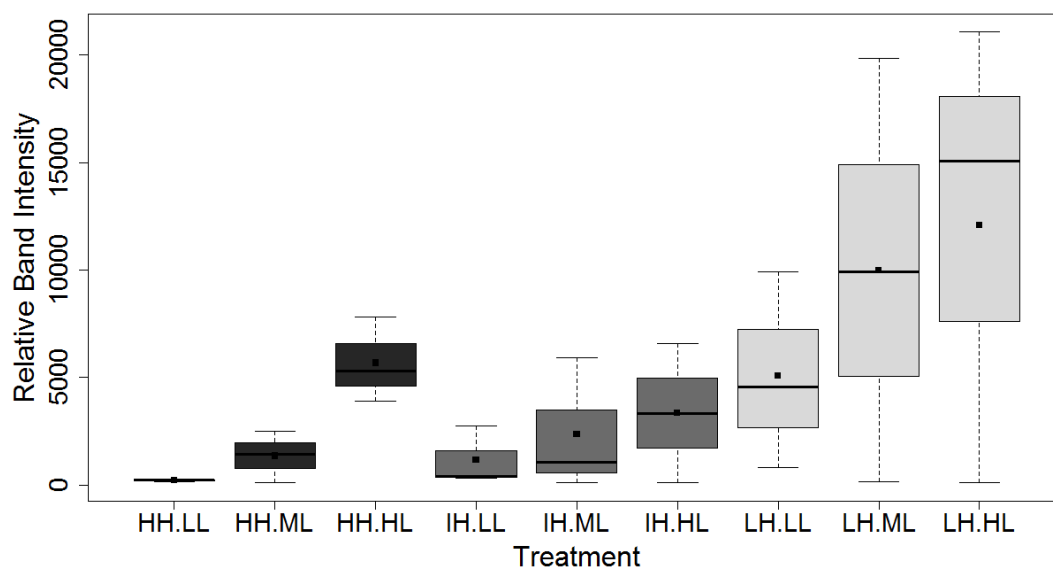


Figure 5.12. Relative intensity of bands representing the 550bp fragment of AM fungal SSU ribosomal RNA gene on Agarose gel from roots from each treatment. HH = High heterogeneity, IH = Intermediate heterogeneity, LH = Low Heterogeneity, HL = High Light intensity, ML = Medium Light intensity, LL = Low light intensity. See methods for details. Black squares are means.

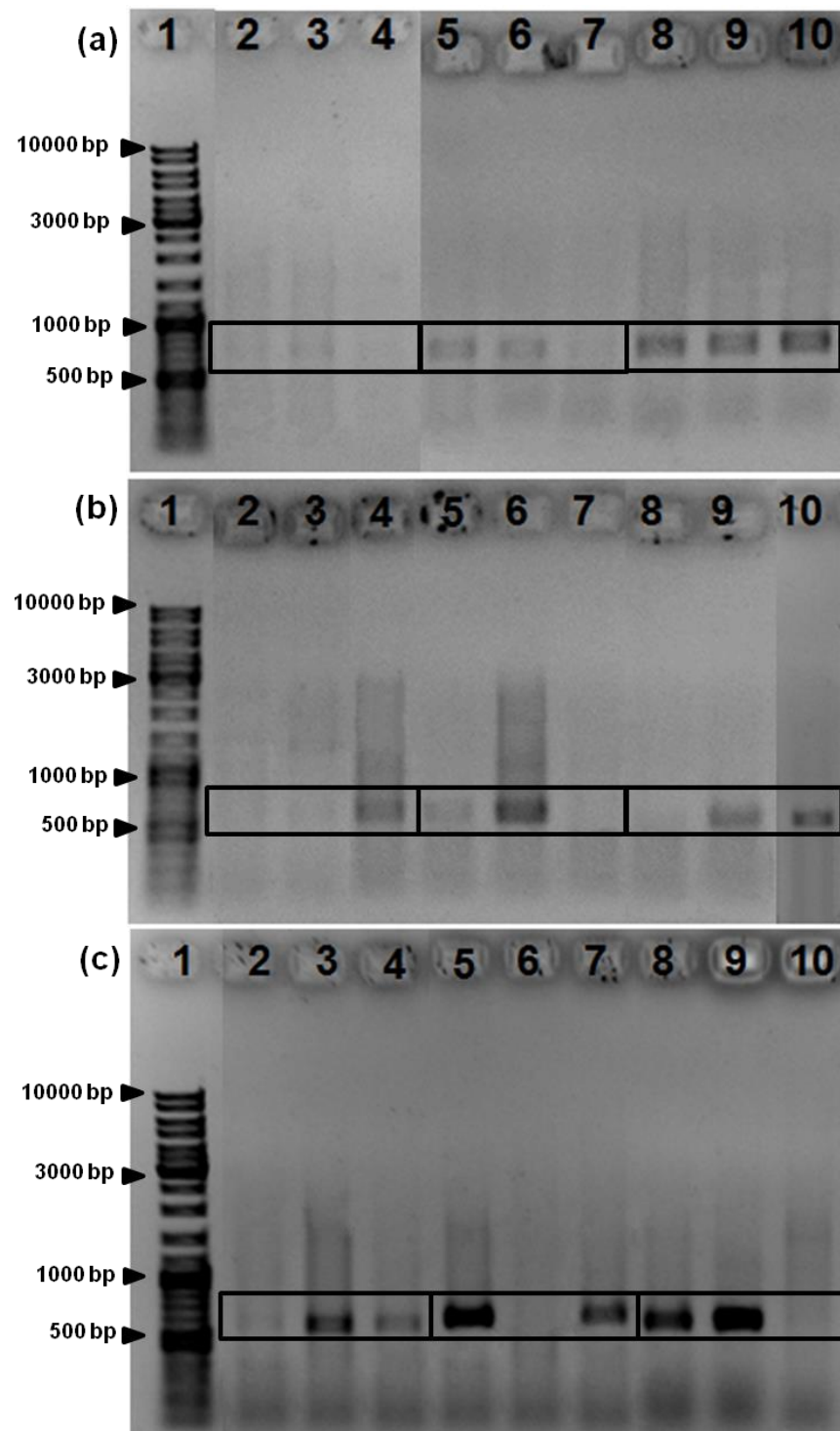


Figure 5.13. 1% Agarose gel with bands representing the 550bp fragment of AM fungal SSU rRNA gene for each heterogeneity and light treatment: (a) High heterogeneity, (b) Intermediate heterogeneity and (c) Low heterogeneity. Lanes 2 - 4 in each gel are in the low light intensity treatment, lanes 5 - 7 are in the medium light intensity treatment and lanes 8 - 10 are in the high light intensity treatment. Image was modified for clarity, although gel band intensity quantification was performed on unmodified image. Sequencing was performed on the Illumina MiSeq platform using a MiSeq reagent kit V3 (2 × 300bp) at TGAC (The Genome Analysis Centre, Norwich).

### 5.5.3 Molecular data

The molecular AM fungal community dataset to be sequenced on the MiSeq platform was not yet available at the time of writing, due to global problems with the Illumina 600-cycle kits.

## 5.6 Discussion

### *5.6.1 Plant physical properties are influenced by energy gradients, and not heterogeneity gradients*

While root biomass, shoot biomass, root:shoot ratio and whole plant biomass all positively correlated with light intensity (Figs. 5.4 - 5.7), the degree of environmental heterogeneity, generated by a range of watering treatments, had no effect on any of these physical properties in *Brachypodium sylvaticum* (Fig. 5.3). Similarly, the water content of the soil had no effect on root, shoot or whole plant biomass (Figs 5.8 - 5.10). However, the root:shoot ratio of those plants with the lowest soil water content (watered to 40% saturation) was significantly greater than those plants watered to 90% and 100% saturation (Fig. 5.11). This result is suggestive of a response to drought in the driest *B. sylvaticum*, as plants tend to increase belowground biomass allocation to improve water and nutrient foraging capacity and to decrease aboveground biomass allocation to decrease water usage and nutrient consumption (Markesteyn and Poorter, 2009, Gargallo-Garriga *et al.*, 2014). This suggests that soil water content is an environmental variable which may have the potential to significantly affect the availability of AM fungal habitat, but did not within the spatiotemporal scale of this experimental design. Any heterogeneity effects on AM fungal community structure, therefore, are more likely to be independent of the host plant. Conversely, but as expected, because the amount of available energy had a

significant effect on the physical properties of the host plant, any effects of energy availability on the associated AM fungal communities is likely mediated by the host plant.

#### *5.6.2 Heterogeneity affects the influence of energy on AM fungal intraradical biomass*

Only in the high heterogeneity treatment did the light intensity make a difference to the intraradical AM fungal biomass (Fig. 5.12). The band intensity in the high light treatment was significantly greater than the low light and medium light intensity treatments. However, a negative correlation was observed between heterogeneity and intraradical AM fungal nucleic acid abundance (used as a proxy for intraradical AM fungal biomass). This suggests that the synergistic effect of heterogeneity and energy on intraradical AM fungal biomass is one in which the greatest AM fungal biomass exists in habitats with the highest available energy but the lowest heterogeneity. For intermediate and low heterogeneity treatments, energy and heterogeneity seem to interact in a manner in which they independently contribute to the biomass (Fig 5.1). Between the lower levels of heterogeneity and the high heterogeneity treatment, however, the positive effect of available energy on AM fungal biomass increases. Given that heterogeneity in soil water content had no significant effect on the physical properties of *B. sylvaticum*, the response of intraradical AM fungal biomass to heterogeneity could be a result of higher heterogeneity limiting root colonisation rates, extraradical growth rates and nutrient exchange with the host plant, or a combination of these. Indeed, soil water content has been observed to have a greater effect on AM fungal spore germination rates than other factors, such as soil fertility, pH and spore density (Daniels and Trappe, 1980). The increased root:shoot ratio in those plants in the driest treatment suggests

that in the high heterogeneity treatment (the only treatment which included the driest plants), carbon allocation to the roots may have been significantly greater in those plants under the high light treatment. Up to 20% of total host plant carbon can be allocated to associated AM fungal communities (Duhamel *et al.*, 2013) and the rate of carbon assimilation in plants is positively correlated with carbon allocation to AM fungi (Lekberg *et al.*, 2013). As such, it is not surprising that when energy provided to the host plant is limited, carbon allocation to its fungal partners is suppressed. The manner in which environmental heterogeneity influences the effect of energy availability on AM fungal communities could have implications for ecosystem services provided by AM fungi, such as carbon sequestration (Treseder and Allen, 2000) and soil stabilisation (Wright and Upadhyaya, 1998). This is due to phenotypic differences among AM fungal species in growth rate and tissue quality, along with the compositional shift often observed in AM fungal communities in response to greater energy availability.

#### *5.6.3 Effects of heterogeneity and energy availability on AM fungal community structure, diversity and composition*

Hiiesalu *et al.* (2014) found that AM fungal richness negatively correlated with both above- and belowground plant biomass. This could be a result of the priority effects so often observed in AM fungi (Dumbrell *et al.*, 2010a) decreasing the likelihood of colonisers with increasing success of the first coloniser and time lag between first and second colonisation, regardless of the identity of the first coloniser (Werner and Kiers, 2015). Indeed, network analysis reveals that there is a greater degree of unevenness in less species-rich communities (chapter 3), which suggests that a very successful first coloniser can suppress growth of other AM fungi. As such, a greater intraradical AM fungal biomass could indicate more species-poor communities.

Thus, the synergistic effect of heterogeneity and energy on AM fungal species richness is likely to produce a cone-shaped relationship. Positive effects on AM fungal richness most likely operate up until the point when any further increase in heterogeneity results in the incorporation of habitats near the limits of the host plant niche. At this point, the limited carbon allocation likely suppresses successful nutrient exchange, potentially resulting in the fungal partners aborting arbuscules in the carbon-poor plant (Javot *et al.*, 2007). This would lead to a species-poor community, as a result of insufficient resources for growth. Therefore, an intermediate degree of heterogeneity would probably support the AM fungal communities with the greatest species diversity. A caveat with these results is the fact that outside of an experimental system, taking into account the heterogeneity of other system properties, the synergistic effects of environmental heterogeneity and available energy on AM fungal diversity are likely to be much more complicated, and potentially unique to each system property. Additionally, because a compositional change in AM fungal communities has often been observed with increased CO<sub>2</sub> (Treseder and Allen, 2000), it is likely that the increase in carbon allocation to the fungal partners with increased energy availability observed in this study produces compositional differences in AM fungal communities under different energy treatments.

The optimal soil water content is similar for AM fungal sporulation and plant growth (Augé, 2001). The relationships between soil water content and plant physical properties observed in this study (Figs 5.8 - 5.11) indicate that this optimum seems to be between 60% and 70% saturation. Thus those plants in the treatments with the smallest degree of heterogeneity, which were all watered to 70% saturation, are likely to host AMF communities in which rates of sporulation are greatest. At such

short temporal scales, however, this is unlikely to significantly affect the AM fungal communities. The response of spore germination rates to a gradient in soil water content, however, is dependent on AM fungal taxon identity (Augé, 2001). Given the similarity of this experimental design to the termination of spore dormancy at the end of winter, AM fungal community composition is likely to be affected by differing degrees of heterogeneity in this soil property in this study. At greater temporal scales, the effects of heterogeneity and energy on diversity are likely to differ. Antoninka *et al.* (2011) observed that increased carbon allocation to AM fungal communities increased AM fungal biovolume, taking into account extraradical hyphal lengths. In turn, the greatest influence on spore richness came from the AM fungal biovolume. After a longer growth period than the one used in this study, this may result in a greater AM fungal diversity in the plants occupying habitats of a lower heterogeneity (which supported the greatest intraradical AM fungal biomass).

#### **5.6.4 Experimental limitations and further work**

As the structure, diversity and composition of AM fungal communities changes throughout time (Bennett *et al.*, 2013), it would be useful to ascertain how the effects of heterogeneity and energy, along with their interaction, influence these changes. In the current study, after only 24 days of growth, large quantities of AM fungal biomass had accumulated inside *B. sylvaticum* plants. As such, it would be interesting and worthwhile to harvest roots and profile the AM fungal communities at earlier and later stages than this. Additionally, to include sporulation, spore germination and root colonisation rates, as well as a molecular profiling of communities in such a long-scale study would yield valuable data regarding the responses of AM fungal community composition and structure to energy and heterogeneity. The low heterogeneity treatment used in this study seemed to subject the plants to ideal



conditions for assimilating carbon, and may have contributed to the much greater AM fungal biomass in the roots of these plants. Using heterogeneity control groups which minimise heterogeneity at a range of values for a particular soil environmental variable (in this case soil water content) could be useful in disentangling the effects of heterogeneity and energy availability.

### **5.6.5 Conclusions**

While the plant physical properties were influenced by the amount of available energy, and not by environmental heterogeneity, intraradical AM fungal biomass was influenced to a much greater degree by heterogeneity. However, root:shoot ratio was greater in those plants in the driest treatment, indicating a response to drought stress. Increased availability of energy only led to significantly greater AM fungal biomass in the high heterogeneity treatment, indicating a suppression of carbon allocation to AM fungal communities in those plants subjected to high heterogeneity and low energy. This combination was evidently the least conducive environment to AM fungal colonisation and growth. It is likely that diversity is lower in those communities with greater AM fungal biomass, due to priority effects increasing unevenness, and that AM fungal diversity peaks in habitats of intermediate heterogeneity and energy. It is clear from this experimental study that the effects of environmental heterogeneity and available energy on AM fungal communities are relatively intractable; as such much further work is required to determine the mechanisms and processes that control the observed patterns.

## Chapter 6: General Discussion

### 6.1 AM fungal host plant preference is driven by plants, soil and AM fungi

The degree of compositional difference between AM fungal communities hosted by different plant species is positively correlated with spatial scale (Chapter 2). Host plant preference is therefore more likely to be detected at larger spatial scales. Indeed, non-random assemblages of AM fungi have been detected more frequently at large spatial scales (Sýkorová *et al.*, 2007b, Hazard *et al.*, 2013) than at small spatial scales (Öpik *et al.*, 2003, Santos *et al.*, 2006). Spatial scale of sampling also influences patterns of diversity, with qualitative differences in AM fungal diversity in different plant species between large and small spatial scales. The aboveground spatial patterns of host plants differ between ecological guilds, and not between individual plant species. The grasses, which were negatively spatially autocorrelated and covered a greater proportion of their experimental plots than the forbs, supported the most species-rich AM fungal communities. The least species-rich AM fungal communities were detected in the forb plant species which displayed the greatest degree of positive spatial autocorrelation and was non-rhizomatous (Chapter 2). Root architecture of individual plant species influences the diversity of AM fungi by determining how much of the belowground environment can be exploited by the plant. This, in turn directly affects the availability of AM fungal habitat and therefore the capacity of the environment to support the coexistence of AM fungal taxa. While a negative effect of fine root biomass on AM fungal colonisation has been observed in subtropical tree species (Liu *et al.*, 2015), the difference between tropical and temperate soils in nutrient availability (Martinelli *et al.*, 1999), means that the opposite is likely to be the case in temperate soils. Due to

species-specific niche space, variation in the soil physical properties of rhizosphere soil exists between different host plant species (Chapter 4). Additionally, given the relatively conserved rank order of AM fungal OTUs between communities, species-specific vital rates certainly constitute a major influence on community structure (Chapter 3). It is likely a combination of interspecific variation in plant root architecture, soil physical properties and AM fungal vital rates that contributes to the detection of host plant preference in AM fungi.

## **6.2 Interspecific phenotypic differences among AM fungi affect community structure**

Nestedness analysis on AM fungal metacommunities revealed no correlation between occupancy and abundance. This is indicative of a phenotypic trade-off in AM fungi between dispersal ability and growth rate. Direct evidence for such trade-offs has been recorded (Mikkelsen *et al.*, 2008, Helgason and Fitter, 2009), supporting the conclusion drawn from AM fungal metacommunity network analysis in the current thesis. That the rank order of AM fungal OTUs did not differ between the majority of communities, suggests that niche-based processes influence community structure more than neutral processes, and that phenotypic differences between AM fungal OTUs determine the abundance of each OTU within the community. The relative influence of AM fungal phenotype and “site quality” was affected by spatial scale, and differed depending on depth of sampling. Given the findings that belowground root biomass and AM fungal diversity are positively correlated (chapter 2), and that heterogeneity negatively affects AM fungal colonisation and growth (chapter 5), greater site quality can be inferred to relate to increased belowground coverage of host plant roots and an intermediate level of heterogeneity.

### **6.3 Effect of environmental heterogeneity on AM fungal communities depends on the parameter**

Variability in numerous environmental variables can be the source of environmental heterogeneity as perceived by AM fungi. Being an obligate endosymbiont with an extensive extraradical mycelium, AM fungi are subject to the physical properties of both the soil and their host plant. The effect of each of these properties, and therefore their variability, is likely to be idiosyncratic. A negative heterogeneity-diversity relationship was observed when the property in question was availability of habitat (chapter 2). High heterogeneity in the spatial patterns of the host plant limits the diversity of their associated AM fungal communities. This is likely due to the resultant fragmentation of the available habitat, leading to effective islands, in which an overdominance by a certain few taxa suppresses the growth of other taxa within the same plant, leading to less diverse communities (Laanisto *et al.*, 2013). Heterogeneity in another environmental variable known to affect AM fungi, soil water content, negatively correlates with intraradical AM fungal biomass (chapter 5). The manner in which intraradical AM fungal biomass affects AM fungal diversity will determine how heterogeneity in soil water content affects AM fungal diversity. It is likely a hump-shaped relationship in which diversity levels peak at intermediate heterogeneity. This is because environments with high heterogeneity may incorporate habitats whose extremes of variables are not conducive to AM fungal growth and colonisation, and in environments with a low heterogeneity, if conditions for plant growth are optimal, high energy availability allows overdominance and thus promotes unevenness in AM fungal communities due to suppression of growth of late-colonising AM fungi. The degree to which heterogeneity contributes to the structure of AM fungal communities in natural environments, however, is still largely

unresolved. The variability of certain important soil environmental parameters, namely organic carbon content, bulk density, pH and root biomass, all of which have been observed to have a major effect on AM fungal communities, did not differ between the local habitat surrounding *Leucanthemum vulgare* and *Festuca rubra* (chapter 4). Plant physical properties and absolute values of these soil variables, however, did. These may drive differences in community composition between host plant species.

#### **6.4 Availability of energy affects structure and diversity of AM fungal communities**

The availability of energy influences physical properties of the host plant (chapter 5). Given the total dependence of AM fungi on their host plant, anything that has a major effect on the host plant's vital rates will inevitably also affect their fungal partners. Indeed, there is a positive relationship between energy availability and intraradical AM fungal biomass. This relationship is strongest when heterogeneity is greatest; indicating that carbon allocation to AM fungi is suppressed in highly heterogeneous environments that incorporate habitats nearing the limit of the host plant's tolerance. In AM fungal communities with more biomass, it is likely that diversity is limited, due to the aforementioned priority effects favouring the primary coloniser of the plant root. Such a positive feedback mechanism which results in greater unevenness in less species-rich communities is evident from the simultaneous qualitative nestedness and lack of quantitative nestedness observed in AM fungal metacommunities.

## **6.5 Further work**

Future studies of host plant preference in AM fungi should incorporate the spatiotemporal structure of the AM fungal habitat, including root architecture, biomass and phenology, along with other belowground environmental variables, to determine their effect on the perceived host plant preference. Experimental studies which profile AM fungal communities at different points throughout a time-series would provide insight into the phenotypic differences between AM fungal OTUs and thus inform models of community structure. Manipulation of the carbon supply to sequentially harvested mycorrhizal plants would be valuable in resolving the temporal dynamics of the relationship between energy availability and diversity and unevenness of communities. Finally, experimental studies which control for host plant identity but vary the spatial autocorrelation of the plant individuals could provide further information about the relative roles of root architecture and host plant identity, and test the hypothesis that the effect of the latter is mediated by the former.

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